Lincoln University
Te Whare Wānaka o Aoraki
CHRISTCHURCH•NEW ZEALAND

Lincoln University Digital Thesis

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.

VECTORING OF *BOTRYTIS CINEREA* (PERSOON: FRIES) TO KIWIFRUIT (*ACTINIDIA DELICIOSA*) FLOWERS BY HONEY BEES (*APIS MELLIFERA* LINNAEUS)

A thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science at Lincoln University

by

E. A. F. Rose

1996

FRONTISPIECE: A honey bee foraging on a staminate kiwifruit flower.

Abstract of a thesis submitted in partial fulfilment of
the requirements for the Degree of M.Sc.

VECTORING OF *BOTRYTIS CINEREA* (PERSOON: FRIES) TO KIWIFRUIT (*ACTINIDIA DELICIOSA*) FLOWERS BY HONEY BEES (*APIS MELLIFERA* LINNAEUS)

E. A. F. Rose

Botrytis cinerea Persoon ex Fries causes stem-end rot of kiwifruit (*Actinidia deliciosa*) in storage. The honey bee (*Apis mellifera*) may influence the epidemiology of *B. cinerea* in kiwifruit orchards by acting as a vector of viable *B. cinerea* propagules to kiwifruit flowers during foraging.

The ability of honey bees to vector viable *B. cinerea* propagules to kiwifruit flowers was assessed. Methods were developed for collecting foraging honey bees directly from flowers and isolating viable *B. cinerea* propagules from the external surfaces of bees. Eighty seven percent of honey bees foraging on kiwifruit flowers carried viable *B. cinerea* propagules. The mean number of propagules carried by bees increased from early to late kiwifruit flowering. Bees carried similar numbers of propagules when foraging on staminate and pistillate kiwifruit flowers.

Honey bees which visited manuka (*Leptospermum* spp.) flowers in close proximity to the experimental kiwifruit orchard site were also contaminated with *B. cinerea*. This demonstrated the potential for foraging bees to transfer inoculum to kiwifruit flowers from external sources.

Honey bees acquired large numbers of dry *B. cinerea* spores (mean c. 4×10^3) while foraging on kiwifruit flowers that had been artificially contaminated with the fungus. The acquisition of spores from artificially inoculated flowers provided evidence that honey bees may pick up spores from naturally infested kiwifruit flowers during pollen collection.

Measurement of the dispersal of *B. cinerea* propagules between kiwifruit flowers showed that honey bee dispersal is an important mechanism for the spread of inoculum over short distances. The dispersal of inoculum by honey bees followed a negative exponential gradient with distance from the inoculum source. Dispersal of *B. cinerea* spores by honey bees was considerably greater than dispersal by wind over short distances.

Application of *B. cinerea* spores to fruit at petal fall stage increased the number of *B. cinerea* propagules on the surface of fruit during the season. This indicated that inoculum spread to flowers by honey bees could influence the contamination of fruit later in the season. High natural levels of *B. cinerea* on fruit surfaces were found, with a general increase in the number of viable propagules per fruit from petal fall stage to mature fruit stage.

The implications of these results for *B. cinerea* epidemiology in kiwifruit orchards are discussed and requirements for further research are identified.

Key Words: Kiwifruit; *Actinidia deliciosa*; stem-end rot; *Botrytis cinerea*; honey bee; *Apis mellifera*; vector; epidemiology.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

Stem end rot of kiwifruit [*Actinidia deliciosa* (A. Chevalier) C. F. Liang *et* A. R. Ferguson (Ericales: Actinidiaceae)] is caused by the fungus *Botrytis cinerea* Persoon ex Fries (Heliotales: Sclerotiniaceae). Brook (1990a) emphasised the need to identify inoculum sources and their mechanisms of spread to improve understanding of *B. cinerea* epidemiology in New Zealand kiwifruit orchards. Fermaud *et al.* (1994) pointed out that little was known about the relevance of kiwifruit flower infection and the role of insect transmission of *B. cinerea* inoculum in the progression of stem-end rot epidemics. In this thesis it is proposed that the honey bee [*Apis mellifera* (Linnaeus) (Hymenoptera: Apidae)] may be an important vector of *B. cinerea* propagules to kiwifruit flowers.

The remainder of Chapter One provides a context for the following five chapters. The epidemiology of *B. cinerea* is discussed in relation to the export kiwifruit production system, the importance of honey bees in the production system, and some ecological aspects of relationships between flower-visiting insects, plant pathogens, and hosts.

1.1 CONTEXT OF THESIS

Kiwifruit Industry Background

Kiwifruit was introduced to New Zealand from China in 1904 (Ferguson & Bollard 1990). The first fruits were obtained in 1910, but the crop was only of local interest until its export potential was realised during the 1960's (Donovan & Macfarlane 1984). The cultivar 'Hayward' is essentially the only pistillate kiwifruit cultivar grown for export fruit production in New Zealand and in most other parts of the world. Kiwifruit is now established as New Zealand's largest horticultural export earner, accounting for 31% of all horticultural exports by value in 1993, compared with apples (25%), vegetables (26%), floriculture (4%) and other fruit (14%). In 1994, the total area of kiwifruit plantings was 10161 ha, with exports of approximately 221 000 tonnes valued at NZ $381 million (Anon 1995).

The New Zealand Kiwifruit Marketing Board (NZKMB) is a government-legislated marketing authority which controls virtually all sales of export kiwifruit, except for a small amount of second grade fruit sold to Australia under the 'Closer Economic Relations' policy. In 1994, New Zealand produced 28% of the total global kiwifruit production. Other major producers were Italy (30%), Chile (10%), France (8%), Greece (5%), USA (5%), and Japan (7%) (Anon 1995).

Botrytis Stem-End Rot Of Kiwifruit

During the early years of commercial development of New Zealand kiwifruit orchards in the late 1960s and 1970s, the crop was regarded as virtually disease-free. With the increasing duration of kiwifruit monoculture, however, the rapid expansion of production, disease problems have become more numerous and important (Pennycook 1985). Stem-end rot induced by *B. cinerea* was first recognised in New Zealand during the 1977-78 season (Beever 1979) and has since become a major cause of post-harvest fruit loss. Kiwifruit stem-end rot has also been reported in Italy (Bisiach *et al.* 1984) and California (Opgenorf 1983; Sommer & Suadi 1984).

In New Zealand, direct losses of export graded fruit vary considerably between sites and seasons, with annual losses of about 0.2-2% of total production, although losses for some individual growers can exceed 30% (Pennycook 1985). The direct costs of stem-end rot are mostly due to the need to repack a much larger proportion of the stored fruit trays during pre- and post-export checking procedures (Poole & McLeod 1992). In the 1994 season losses due to stem-end rot were NZ $7.18 million onshore and NZ $8.31 million offshore (Burt pers. comm.). The tolerance level set for stem-end rot before pallets of trays must be repacked is two fruit per 400 fruit checked (0.5%).

***Botrytis cinerea* Host Range And Taxonomy**

B. cinerea is a widespread facultative parasite producing infections on more than 200 hosts including dicotyledons, monocotyledons, gymnosperms, and ferns (Coley-Smith *et al.* 1980; Beever & Parkes 1993; Jarvis 1977). In New Zealand, *B. cinerea* affects a wide range of economically important crops, including kiwifruit, grapes, berryfruit, vegetables and ornamentals (Pennycook 1989).

Botrytis was one of the first described genera of fungi, erected by Micheli in 1729, and validated by Persoon in 1801 and Fries in 1832 (Jarvis 1980a). *B. cinerea* is the asexual (anamorph) binomial for *Botryotinia fuckeliana* (de Bary) Whetzel (teliomorph or sexual phase). This teleomorph is also often referred to as *Sclerotinia fuckeliana* (de Bary) Fuckel. Based on the anamorphic classification, the genus *Botrytis* is placed in the family Moniliaceae, order Hyphomycetales, class Hyphomycetes and sub-division Deuteromycotina (Ainsworth *et al.* 1971). Jarvis (1980a) has reviewed other methods of classifying *Botrytis* based on conidiogenesis and numerical techniques. Based on the teleomorphic classification, *B. fuckeliana* is placed in the family Sclerotiniaceae, order Heliotales, class Discomycetes and sub-division Ascomycotina (Korf 1973). The true taxonomic position of species of *Botrytis* and *Botryotinia* remains largely unresolved due to confusion and controversy over generic limits in the family Sclerotiniaceae (Kohn 1979). Strictly, the organism causing stem end rot of kiwifruit should be referred to by its holomorph name - *B. fuckeliana* (Hennebert & Weresub 1979), or the *Botrytis* state of *B. fuckeliana* (Ellis 1971), but because of common usage and convention, it will be referred to in this thesis by its anamorph binomial - *B. cinerea.*

Epidemiology Of *Botrytis cinerea* In Kiwifruit Orchards

It is now widely agreed that the majority of kiwifruit stem-end rot infections are initiated during harvest, grading and packing operation, by *B. cinerea* propagules infecting the fruit via the picking wound that is formed where the fruit is snapped from its pedicel (Pennycook 1985). A summary of current understanding of the epidemiology of *B. cinerea* through the year in New Zealand kiwifruit orchards follows, including postharvest disease etiology.

The form, function and pathology of *B. cinerea* have been described in detail by Coley-Smith *et al.* (1980) and Jarvis (1977). High humidity and temperatures of 15-20°C favour spore germination, infection, growth, and sporulation of *B. cinerea* on senescent and necrotic kiwifruit tissues (Brook 1992).

Winter (June-September)

B. cinerea survives as a perthophyte of kiwifruit (i.e., is able to some extent, to kill host tissues in advance and obtain nutrients from the dead cells) (Anon 1973). *B. cinerea* also produces

sclerotia which can overwinter and produce conidia and apothecia in the following spring and summer (Henis *et al.* 1987).

Elmer *et al.* (1992; 1993; 1994) identified major *B. cinerea* inoculum sources in Motueka kiwifruit orchards by measuring the incidence and area of lesions on host tissues. Necrotic canes pruned during June-July, and left on the orchard floor, were primary sites of inoculum production from the start of sampling in September through to the beginning of the flowering period (late November). Two weed species, the broad leaved dock (*Rumex obtusifolius*) and willow weed (*Polygonum persicaria*) were also sites for overwintering and oversummering survival of *B. cinerea* (Elmer *et al.* 1992).

Spring-Summer (October-March)

Elmer *et al.* (1992) detected *B. cinerea* in leaf litter under vines and on a small proportion (<5%) of female flower buds before the flowering period. The incidence of infection on necrotic winter-pruned canes declined from flowering to harvest due to the decay of canes on the ground over time (Elmer *et al.* 1992).

During late blossom and petal fall, *B. cinerea* often becomes conspicuous, and profuse sporulation may be visible on 80-90% of blossoms with senescing petals (Pennycook 1985). A significant correlation between the incidence of *B. cinerea* flower infections and stem-end rot in storage has been demonstrated (Elmer *et al.* 1992). Flower infections may influence stem-end rot indirectly by increasing the contamination of fruit surfaces with adhering infected flower tissues (Elmer *et al.* 1992) and increasing the amount of inoculum in orchards (Elmer *et al.* 1992; 1994). Elmer *et al.* (1992; 1994) showed a distinctive peak in spore numbers trapped from orchard air at petal fall compared to other growth stages. The significance of latent fruit infections initiated during flowering has not been elucidated (Fermaud & Gaunt 1995), but may influence the amount of distal-end rot in storage (see *Latent Infections* below).

At petal fall, large spreading leaf lesions may develop from secondary spread via adhering debris from infected blossoms (Pennycook 1985). These infections are assisted by prolonged leaf wetness and pollen as a source of easily-degradable nutrients, such as amino acids and sugars (Clark & Lintas 1992).

Although leaf disease does not affect vine vigour, rain wash from damaged leaves can cause surface staining of fruit (Brook 1990b) which can result in rejection of fruit for export. Pruning of staminate vines immediately after petal fall results in a large mass of necrotic leaves and canes, most of which are mulched on the orchard floor, but some debris remains lodged in the growing canopy, adding to the mass of tissues available for infection. During summer months, up to 100% of necrotic leaves and senescent shoots may be infected (Elmer *et al.* 1994).

Autumn Harvest (April-May)

There are many potential sources of *B. cinerea* inoculum available for infection of stem-wounds induced by kiwifruit harvesting procedures. Evidence suggests that these inoculum sources are more important, in terms of kiwifruit infection, than those present earlier in the season. At harvest necrotic areas on green leaves are a principle source of inoculum although spore numbers trapped from the air are low relative to other periods in the growing season (Elmer *et al.* 1992). Manning & Pak (1993) reported a high correlation between pre- and post-harvest incidence of *B. cinerea* on leaf discs and incidence of storage rots. Hoyte *et al.* (1994) compared *B. cinerea* colonisation and inoculum production on necrotic leaf tissue from lightly and heavily pruned orchards. Both colonisation and spore production were significantly higher in the lightly pruned orchard, and led to a higher incidence of storage rot in fruit compared to the heavily pruned orchard. *B. cinerea* colonised a greater proportion of necrotic tissue on green leaves with necrotic lesions than on completely necrotic leaves, indicating that on necrotic lesions the fungus has the ability to pre-dominate, while on dead leaves it is present only as a saprophyte (Hoyte *et al.* 1994). Removal of necrotic leaves from orchards markedly reduced the overall *B. cinerea* population, with the effect being most noticeable in the postharvest period (Pak & Manning 1993).

During handling after harvest, contamination may occur through direct contact of the picking wound with an inoculum source, such as infected sepals attached to fruit or plant debris. Each piece of debris can hold large numbers of infectious agents (hyphae, spores and germlings) of *B. cinerea*, and all these agents have been found to be capable of producing rots (Hallet & Sharrock 1993). *B. cinerea* propagules may also be present on the external surface of kiwifruit (Fermaud *et al.* 1994). Contamination of the picking wound with such inoculum sources increases while fruits are jostled in picking bags (Brook 1990a; Elmer *et al.* 1994; Pak & Manning 1993) and

the number of viable *B. cinerea* propagules on the fruit surface at harvest has been significantly correlated with subsequent stem end rot in storage (Elmer *et al.* 1995).

Stem-End Rot Infection And Symptoms In Cool Storage

B. cinerea spores germinate in the uppermost ruptured cell layers of the stem wound, and penetrate most rapidly via the vascular elements, with hyphae growing into the loosely packed pericarp and core tissues. Although the optimum temperature for *B. cinerea* spore germination and mycelial growth is 15-20°C, in cool storage (0°C) the fungus is still capable of spore germination and mycelial growth giving it a competitive advantage over many other fungi on kiwifruit (Bisiach *et al.* 1984; Manning & Brook 1991).

The first symptoms of stem-end rot of kiwifruit typically begin to appear after four weeks of cool storage (Pennycook 1985), with no further incidence found after 10-12 weeks of storage (Brook 1990a). The typical delay between infection and appearance of rot symptoms in the body of the fruit is due to relatively slow, steady growth of hyphae through dense tissues of the stem plug (Hallet & Sharrock 1993; Hallet *et al.* 1991; Sharrock & Hallet 1992). As the rot advances from the stem towards the fruit's distal end, the diseased flesh appears glassy, watersoaked and darker than healthy tissue. After several weeks, the rot may spread throughout the fruit, but often the distal end remains unaffected (Pennycook 1985). At an advanced stage, fluffy mycelia, at first white, then becoming grey with the development of conidiophores, emerge from the rotted fruit. Mycelia may spread to adjacent healthy fruits causing secondary infections ('nesting'), which are detectable after 10 weeks in cool storage (see Figure 1-1). (Brook 1990a, 1990b).

Figure 1-1: Kiwifruit with stem-end rot in storage.

Ethylene gas produced by fruit infected with *B. cinerea* during storage increases the rate of fruit softening/ripening, and also increases the amount of ripe rots in fruit at ambient temperatures after storage (Brook 1990a). *B. cinerea* infections that lead to storage rot can also be initiated at wound sites on the fruit surface (Brook 1992). Anecdotal reports suggest that *B. cinerea* rot can develop on surface bruises obtained through rough handling of fruit during packing (Rose pers. comm.). Other fungi which cause 'ripe' or 'breakdown' rots after removal from cool storage are components of the microflora of kiwifruit orchards, such as species of *Botryosphaeria*, *Fusicoccum*, *Glomerella*, *Colletotrichum*, *Cryptosporiopsis*, *Diaporthe*, *Fusarium*, and *Phoma*, but do not cause disease at 0°C (Brook 1992).

Latent Infections

At harvest, *B. cinerea* infections may be established within fruit tissues in a latent form (Sommer & Suadi 1984). Elmer *et al.* (1992) demonstrated the presence of latent *B. cinerea* infections in kiwifruit at petal fall and mid fruit development, while Bisiach *et al.* (1984) isolated *B. cinerea* from the pulp of healthy cool stored fruit.

There is wide support for the hypothesis that, in fruits such as tomato (Lavey-Meir *et al.* 1988), grape (McClellan & Hewitt 1973; Nair 1985), raspberry (McNicol *et al.* 1985), and blackcurrant (McNicol & Williamson 1989), mycelia of *B. cinerea* latent in immature fruit can renew growth

and cause rot when the physiological changes associated with ripening occur. Despite the demonstration of latent infections in kiwifruit at several growth stages (Bisiach *et al.* 1984; Elmer *et al.* 1992), it has not been shown whether they develop into aggressive rots.

Latent infections may contribute to the incidence of distal-end rots, which commonly make up less than 5% of *B. cinerea* rots in storage (Sommer & Suadi 1984); or they may remain symptomless until fruit is removed from coolstorage, when they may contribute to ripe rots (Brook 1992).

Management Of Botrytis Stem-End Rot

Successful management of stem-end rot directly affects export market access and grower revenue. Since 1979 the protectant dicarboximides, vinclozolin (Ronilan) and iprodione (Rovral), have been applied to export crops (Brook 1990a). The New Zealand kiwifruit industry is currently working towards a 'Kiwigreen' spray programme for pest and disease control which focuses on no detectable residues being found on the fruit at the point of sale (Smith 1993). Growers in this programme are unable to apply a pre-harvest dicarboximide spray. In 1995, 40% of export fruit was grown under the Kiwigreen concept (Burt pers. comm.). Non-'Kiwigreen' growers can apply a single dicarboximide spray as little as 24 h prior to harvest which may reduce the incidence of stem-end rot and also protect fruit in store from secondary 'nesting' infection (Brook 1990a; 1990b). Unfortunately, pre-harvest sprays do not completely eliminate *B. cinerea* infection due to lack of fungicide coverage of the stem wound infection site. Postharvest dipping of kiwifruit in iprodione can reduce stem-end rot, but such postharvest treatments do not have general international approval (Pyke *et al.* 1994).

B. cinerea strains with resistance to the active ingredients in vinclozolin and iprodione have become established in many New Zealand kiwifruit orchards (Beever & Brien 1983; Beever *et al.* 1989; Pennycook & Manning 1985). Due to the presence of dicarboximide-resistant *B. cinerea* strains and pressure from export markets to reduce chemical residue levels on fruit, interest in alternative disease control strategies which are not based on chemical dependency have increased (Elmer *et al.* 1994; Holland *et al.* 1987). Methods of culturally controlling *B. cinerea* prior to harvest aim to both increase air movement and sunlight into kiwifruit vines so that humidity within the crop canopy is reduced, and to remove senescent material that could be

an inoculum source. Postharvest cultural controls aim to reduce the number of spores infecting the stem wound during the first few hours after harvest. Stem-end rot incidence can be reduced by 'curing' fruit, which involves holding fruit at ambient temperature (optimum 10-20°C) between harvest and grading/packing operations (Pennycook & Manning 1992). The exact mechanisms of defence against *B. cinerea* infection at the stem scar brought about by curing are not known, but curing may enhance the development of an effective defence mechanism (Long & Bautista-Banos 1994) or simply seal the susceptible tissues. Packhouses in New Zealand are now strongly recommended to cure fruit after harvest. Indications in 1995 were that all packhouses did some form of curing for 24-96 hours (Burt pers. comm.).

Kiwifruit Pollination

In studying the role of honey bees as a vector of *B. cinerea*, it is important to review current knowledge of kiwifruit flowering and pollination practices, since bees are key pollinators. Kiwifruit pollination is one of the most important factors determining crop yield, as up to 90% fruit set is required for satisfactory commercial yields (Sale 1985). The most common cause of small and/or mis-shappen, unmarketable fruit is inadequate pollination (Ferguson 1984).

Timing And Duration Of Flowering

Kiwifruit vines are dioecious and need cross-pollination, so pistillate vines are interplanted with staminate vines. In New Zealand, orchardists commonly plant one staminate for every 5-8 pistillate vines. The onset and the duration of pistillate and staminate flowering periods vary between years and between orchards (Hopping 1990). The pistillate cultivar Hayward blooms for 2-6 weeks beginning mid November- early December. The most widely grown staminate cultivar, Matua, tends to start flowering 4-5 days before pistillate vines, then flowers open rapidly to full blossom. Although staminate flower availability is not usually a constraint to pollination, in some seasons, anecdotal evidence suggests that staminate vines may reach full bloom while female vines are at only 10% bloom. After full bloom, lateral staminate flowers extend the flowering period, which often ends 3-7 days after pistillate vines have finished (Alexander 1986).

Pollen dehiscence

Kiwifruit flowers are borne laterally and hang within or below the canopy. This lateral position of the flowers is found in only a few other fruiting plants, such as avocados, mulberries, figs and persimmons (Ferguson 1984). Both staminate and pistillate kiwifruit flowers produce pollen but neither produce nectar. Pollen from pistillate flowers is not viable and contains about 15% protein, whereas staminate pollen is viable and contains 40-45% protein (Clark 1990). Anthers from both staminate and pistillate kiwifruit flowers dehisce by a longitudinal split in the anther wall (Goodwin 1986a). With pistillate anthers, the split opens about 1/5 of the way down on the first day the flower opens (Goodwin 1986a). The split lengthens each successive morning until anthers are completely open on the fifth morning (Goodwin 1986a). Pistillate pollen adheres in clumps to the anther walls so that it has to be scraped off by foraging honey bees (Goodwin 1986a). Only pollen that has been exposed by the splits is available for collection (Goodwin 1986a). The anthers on the staminate flowers liberate dry, powdery pollen for 2-3 days after flowers open (Goodwin 1986a) which can become airborne (Clinch & Heath 1985; Craig & Stewart 1988). Pistillate stigmas are receptive to staminate pollen and can set full-size fruit up to seven days after pistillate flowers open (Goodwin & Steven 1993). Although petals and stamens may turn brown and fall before pollen liberation has ended, flowers are still visited by honey bees for as long as they have pollen available (Goodwin & ten Houten 1989). Wind alone does not pollinate sufficient flowers to regularly produce good crops of export-sized fruits (Donovan & Macfarlane 1984).

Honey Bee Pollination

In New Zealand, honey bees are by far the most important pollinators of kiwifruit because they enormously outnumber all other pollinating insects and because they are fully manageable (Donovan & Macfarlane 1984). Honey bees alone provide many kiwifruit growers with a cost-effective pollination system, although some orchardists may use artificial pollination as well as honey bees (Bryant 1991). New Zealand kiwifruit growers use up to eight hives per hectare (c. 160 000 foraging honey bees) to ensure adequate pollination (Bryant 1986). This number, which is higher than for most other crops, is advised for several reasons. First, the kiwifruit flower is relatively unattractive to bees. Second, bees are often constant on one sex of flower (described in the following section) and are, therefore, not effective pollinators. Third, plant

species providing alternative pollen and nectar sources flower at the same time as kiwifruit (e.g., clover, citrus, flax and rewarewa) (Sale 1985).

Honey bees are active in kiwifruit orchards between about 0800 h and 1700 h, with peak activity occurring around 1200 h (New Zealand daylight saving time) (Goodwin 1986b). Factors affecting the success of honey bee pollination include weather conditions, quality of beehives, supply of staminate pollen, number of pistillate flowers, duration of overlap between staminate and pistillate flowering, size of orchards, density of female canopy, effects of shelter on foraging behaviour, and the attractiveness of competing flowers (Blanchet 1990; Clinch 1984; Lyford and Underwood 1992).

Honey Bee Discrimination Between Staminate And Pistillate Flowers

Honey bees can discriminate between staminate and pistillate kiwifruit flowers (Goodwin 1986b). Up to 80% of bees may exhibit a preference for visiting pistillate flowers and remain relatively constant to pistillate flowers within a foraging trip (Goodwin & Steven 1993; MacFarlane & Ferguson 1984). Few bees, however, show constancy to staminate flowers (Goodwin & Steven 1993). Honey bees often have a particular foraging area which they return to on consecutive trips and this may also restrict contact to one sex of flower. Where there is a lot of pressure on the supply of pollen, foragers collect both staminate and pistillate pollen within foraging trips. There have been anecdotal reports of higher numbers of honey bees foraging in staminate than pistillate vines (Rose pers. comm.). This may be due to the earlier start to staminate flowering, larger floral displays, ease of collecting dry staminate pollen compared with sticky pistillate pollen, and possible differences in olfactory attractants. Shykoff & Bucheli (1995) showed that insect pollinators of *Silene alba* also preferred plants with large floral displays, and also preferred staminate to pistillate flowers. Contrasting observations of greater numbers of bees foraging pistillate than staminate flowers (Goodwin & Steven 1993), may be due to the greater numbers of pistillate vines, and therefore pistillate flowers, in orchards.

Goodwin & ten Houten (1989) used a video camera to survey groups of flowers during honey bee foraging, and found that many of the flowers received in excess of 30 visits per day. Jay &

Jay (1984) calculated that, under ideal foraging conditions, honey bees visited at least 100 flowers per hour and that each pistillate flower was visited at least 4-6 times per hour.

Insect Involvement In The Dispersal Of Plant Disease Agents

It is often the dispersal phase of pathogen life cycles about which least is known. Understanding dispersal requires research at more than one location, and is often more difficult to study experimentally than other phases (Fitt & McCartney 1986). Spore dispersal can be divided into three interrelated phases: spore removal, dispersal and deposition. Insects may be involved in all three stages.

A wide variety of insects are known to act as vectors of viral, bacterial and fungal plant pathogens. Many viruses are transferred between plants only by insect vectors (Bos 1983; Hill 1984). The degree of dependence and specification between pathogens and insect vectors range from obligate (e.g., circulative and noncirculative virus transmission) to casual (e.g., insect dispersal of naturally wind-dispersed fungal spores, such as *B. cinerea*). The relative efficiencies of these mechanisms differ widely. For example, the efficiency of transmission of *Ceratocystis ulmi* (Dutch elm disease) by bark beetles (*Scolytus* spp.) is low because often the beetle bears no spores, the spores are not rubbed off, are rubbed off in the wrong place to infect, or do not germinate (Holmes 1980). However, the low degree of vector efficiency by an individual beetle is compensated for by the fact that elm bark beetles fly in small swarms and sometimes trees are fed upon by hundreds of beetles (Holmes 1980).

It is proposed in this thesis that honey bees transfer *B. cinerea* spores casually to kiwifruit flowers, as occurs in strawberries (Jarvis 1980b). In the following sections, primary emphasis is placed on the dispersal of fungal pathogens by flower-visiting insects.

The role of insects in the transmission of fungal plant pathogens has seldom been investigated thoroughly. A close association between an insect vector(s) and a fungal plant pathogen has been unequivocally established in only a few cases. Such associations and relationships between a vector(s) and a pathogen have been studied much more extensively in viral, mycoplasmal and some bacterial diseases of plants (Harris 1981). In fungal diseases, the association is most frequently assumed or inferred from limited observations, or from

similarities with other diseases (Agrios 1980). Experimental proof for many statements suggesting transmission of a fungal pathogen by insects is often lacking and the kind of relationship between vector and pathogen is unknown. Furthermore, there is no information on the role of the vector in the survival of the pathogen and on the overall dissemination and severity of the disease in comparison to wind, water, man and other vectors(Agrios 1980).

With viral, mycoplasmal and some bacterial diseases, the insect vector(s) is often the only vector (Harris 1981). In most fungal diseases where an insect can be a vector, however, the insect is often assumed to be less important, than several other vectors (Agrios 1980). This may not be a robust assumption since there are several fungal plant diseases in which insects have already been implicated as the main means of dissemination of the pathogen (Holmes 1980) Insects also play a role in fungal diseases by making wounds through which fungi may enter plants. For example, New Zealand flower thrips, *Thrips obscuratus*, were implicated in the transmission of *B. cinerea* to kiwifruit flowers as well as in the predisposition of flowers to infection (Fermaud *et al.* 1994; Fermaud & Gaunt 1995). The penetration of grape berries and stems by *B. cinerea* seems to be facilitated and increased by the lepidopterans *Lobesia botrana* (Fermaud & Le Menn 1989) and *Argyrotaenia pulcellana* (Agrios 1980).

Role Of Insects In The Transmission Of Diseases Affecting Foliage

Fungi which primarily plant foliage often produce fruiting structures, spores and sometimes mycelia which emit odours attractive to insects. The insects sometimes feed on fungal exudates, spores and mycelium, or on plant sap available from plant tissue rotted by the fungus. These insects may ingest or become contaminated with fungal spores or mycelial fragments which they then carry to other plants, or plant parts, they visit. In some cases, a wide variety of insects may be involved in the transmission of some fungi, while in others, more or less specific associations have been developed between a fungus and one or a few insects that act as vectors.

Role Of Insects In The Transmission Of Diseases (or Fungi) Affecting Buds And Flowers

Insects that visit pathogen-infected or contaminated flowers whilst seeking nectar and pollen are likely to become smeared with spores and mycelium which they may then distribute more widely. In most cases, disease transmission by insects to flowers is incidental to the activities of the insect during feeding and/or pollination. In some cases, insects may ingest spores and

mycelial fragments which retain their viability in the insect and may start new infections if they are deposited on susceptible tissues (Agrios 1980).

Of flower-visiting insects, large and hairy insects, such as bees, flies and moths, have the potential to carry larger numbers of plant pathogenic propagules on their bodies than insects such as aphids and thrips. Larger insects also tend to fly faster and remain active under more adverse weather (Agrios 1980).

Honey bee dispersal of crop pathogens may be an important factor in disease epidemiology where bees are important crop pollinators, especially if flowers are prone to blights and rots. Honey bees, because of their importance as pollinators, have been studied most widely with regard to disease transmission to flowers of pome and berry fruit plants (Jarvis 1980b; Johnson *et al.* 1993; De Wael *et al.* 1990; Boylan-Pett *et al.* 1991). These studies have shown honey bees to be an efficient mechanism for spreading large numbers of inoculum propagules from hives to flowers. Honey bees can also disperse conidia of *B. cinerea* among strawberry flowers (Jarvis 1980b).

Honey bees are one of over 70 insect species associated with spread of the fire blight pathogen, *Erwinia amylovora*, of pome fruit. Although honey bees are considered the most important vector of *E. amylovora* during flowering, many other insects have been implicated as probable casual vectors, including wild bees, wasps, flies, aphids, beetles and thrips (Harrison *et al.* 1980). Honey bees have also been demonstrated to transmit pollen infected with blueberry leaf mottle virus (BBLMV) between plants during pollination. It is not known if the virus is vectored by bees that injure blossom tissues during pollination, thus allowing direct inoculation by deposition of infected pollen in wounds, or by ovule infection following fertilization by infected sperm (Boylan-Pett *et al.* 1991).

Due to their ability to transfer pathogens to flowers, honey bees' ability to transfer biological control agents to flowers is also being studied. Peng *et al.* (1992) demonstrated that honey bees can deliver inoculum of the fungus *Gliocladium roseum* to strawberry flowers for biological control of *B. cinerea*. Honey bees have also been effective at disseminating inoculum of

Pseudomonas fluorescens (Johnson *et al.* 1993; Thomson *et al.* 1992) and *Erwinia herbicola* (Thomson *et al.* 1992) to pome flowers for the biological control of fire blight.

In the absence of flowers, honey bees will gather many other substances as substitutes for pollen, including sawdust, gum from trees, epidermal plant hairs, fine black earth from swamps, coal dust, bran or meal from barns and, at times, spores of fungi (Shaw 1990). The protein levels of some spores may be sufficient to categorise them as a nutrient source for bees (Shaw 1990). This behaviour could contribute to the transfer of some fungal diseases by honey bees by action other than flower pollination. Shaw (1990) reviewed the incidental collection of fungal spores by honey bees in lieu of pollen in some detail, and included observations of honey bees collecting rust spores (including *Melampsora*, *Zaghouania* and *Puccinia* spp.), powdery mildew conidia (*Oidium* sp.) and *Neurospora* from a variety of plant sources.

Consequences For Fungi

A major advantage of biotic (insect) transportation over abiotic (wind and water) transport for fungi is that it is less random with respect to plants, allowing for the exploitation of new hosts. For rust fungi, fertilization can only take place when spermatia (non-motile sperm) of one mating type are carried from one spermagonium to another that has receptive hyphae of the opposite, compatible mating type. The transfer of spermatia, which ooze out of the spermagonium in a sticky, sugary exudate, is accomplished in part by rain water, but most frequently it is accomplished by insects which visit the spermagonia for the sugary exudate, become smeared with spermatia and then transport them to other spermagonia (Agrios 1980). For example, the fungus *Uromyces cladii* is adapted to use *Elachiptera* spp. flies which are host-specific pollinators of *Peltandra* spp. Before *Peltandra* spp. flower, the fungus exudes on the host's leaves spermatia in sugary exudate which smells like the host's flowers. The flies visit and feed on the spermatia and, in the process, aid in outcrossing of the fungus. The flies then act as vectors, transporting the aeciospores to *Peltandra* spp. flowers, where they germinate and infect the new host. This relationship may be mutualistic, as the fungus provides a food source for the host-specific flies before the *Peltandra* flower. This may facilitate population growth of the flies, leading ultimately to more *Peltandra* pollination later. The flies are critical for fertilization and for dispersal of the fungus, and infection causes little reduction in host survival

(Roy 1994). More than a hundred species of insects including flies, bees and ants that visit rust spermagonia and, presumably, transmit spermatia, have been observed (Agrios 1980).

1.2 AIM AND OUTLINE OF THESIS

This thesis represents work that commenced in March 1992 under the supervision of Dr Roy Gaunt, Mr Bruce Chapman and associate supervision of Dr Phil Elmer. The aims of the thesis were to determine whether honey bees carry viable *B. cinerea* propagules in kiwifruit orchards, to examine their ability to acquire inoculum from kiwifruit flowers and vector the fungus between kiwifruit flowers, and to assess the relationship between fruit surface contamination by *B. cinerea* at petal fall stage with fruit contamination during the growing season.

The thesis comprises five further chapters that investigate the honey bee vector process and *B. cinerea* epidemiology as follows:

Chapter Two: B. cinerea *As Part Of The Natural External Mycoflora Of Honey Bees Foraging On Kiwifruit Flowers,*

(i) *Investigating methods for capturing honey bees;* The development of a honey bee collection technique is described.

(ii) *Do honey bees carry viable* B. cinerea *propagules?*: The assumption that honey bees carry viable *B. cinerea* propagules naturally in kiwifruit orchards is tested by isolating the fungus from the surfaces of foraging honey bees.

(iii) *Variation in the number of* B. cinerea *cfu carried by honey bees at early, mid and late kiwifruit flowering;* The mean number of viable *B. cinerea* propagules carried naturally by honey bees foraging on staminate kiwifruit, pistillate kiwifruit and manuka flowers is quantified at early, mid and late stages of flowering.

Chapter Three: *Acquisition Of* B. cinerea *Spores From Kiwifruit Flowers By Honey Bees;* The ability of honey bees to pick up *B. cinerea* spores from kiwifruit flowers while foraging is assessed by applying spores to flowers, collecting bees foraging on these flowers, then isolating *B. cinerea* from the surface of bees.

Chapter Four: *Transfer Of* B. cinerea *Between Kiwifruit Flowers By Honey Bees;* The number of *B. cinerea* propagules transferred by honey bees and wind to kiwifruit flowers with distance from a source of flowers contaminated with *B. cinerea* spores is examined.

Chapter Five: *Relationship Between Surface Contamination of Fruit by* B. cinerea *at Petal Fall with Mid Fruit and Mature Fruit Stages;* The significance of *B. cinerea* contamination of fruit during early development at flowering, to fruit surface contamination at mid and mature stages of development is investigated. Surface contamination of fruit with *B. cinerea* was manipulated at petal fall stage by applying different numbers of spores to fruit, then the number of cfu per fruit were measured one and four months later.

Chapter Six: *General Discussion.*

2. *B.CINEREA* AS PART OF THE NATURAL EXTERNAL MYCOFLORA OF HONEY BEES FORAGING ON KIWIFRUIT FLOWERS

2.1 INTRODUCTION

The incidence of kiwifruit flower infection by *B. cinerea* increases from early bloom to petal fall as the mass of necrotic and senescent flower tissue, available as a food base for the fungus, increases (Elmer *et al.* 1992). Honey bees may facilitate in the transfer of *B. cinerea* propagules from infected to healthy flowers while foraging, thus contributing to increased inoculum production in orchards at flowering through establishing secondary infections. An increase in *B. cinerea* inoculum production at flowering may lead to an increase in inoculum availability for picking-wound infection at harvest. Flower infection can also lead to latent infections of fruit which may become aggressive in storage.

Honey bees are reasonably dedicated to foraging one sex of kiwifruit flower within, and sometimes between, each foraging trip from the hive (Section 1-2). If honey bees pick up infective *B. cinerea* propagules from the flowers they forage, then the number of propagules they carry could be related to the stage of kiwifruit flowering, the incidence of disease on the flowers of each sex, the fungal stage presen, and the extent of contact of the bees with propagules during foraging.

The first aim of this chapter was to develop honey bee collection and *B. cinerea* detection techniques. These are described in Section 2.2.1. The null hypothesis (H_0) that honey bees carry viable *B. cinerea* propagules while foraging kiwifruit flowers is tested in Section 2.2.2. This hypothesis was supported and further experiments described in Section 2.3 were conducted to investigate temporal variation in numbers of *B. cinerea* propagules carried by honey bees foraging on staminate and pistillate kiwifruit flowers and manuka (*Leptospermum scoparium*) flowers.

2.2 INVESTIGATING METHODS FOR CAPTURING HONEY BEES

The following criteria were used to develop a method for capturing honey bees suited to the aims of this study: (i) Bees must be foraging kiwifruit flowers; (ii) Bees needed to be sampled individually; (iii) Many bees needed to be sampled in a day; (iv) The risk of *B. cinerea* propagules being dislodged from bees and lost from samples had to be minimal; (v) There had to be minimal reduction in the viability of *B. cinerea* propagules.

2.2.1 METHODS

Study Site

Honey bee capture methods were trialled in a mature, 0.5 ha commercial kiwifruit orchard (cv. 'Hayward') located at the Riwaka HortResearch Research station, near Motueka (41°06.8' South, 173°00.6' East) in the South Island of New Zealand. Vines in the orchard were trained on a T-bar system (1.8m high x 2m wide) with rows 5m apart and with a 1:8 staminate:pistillate vine ratio.

Collection Of Honey Bees

Methods of capturing honey bees were investigated during the November-December 1992 kiwifruit flowering period. Several approaches to honey bee collection were tested as follows: (i) Bees were captured at the hive both by sweep-netting and by trapping bees in vials or with tweezers on hive landing boards; (ii) Bees were sprayed with the aerosol insecticide Slay (tetramethrin 3.3g/l, D-phenothrin 0.8g/l, piperonyl butoxide 7.7g/l) while they foraged, then transferred into vials. The viability of *B. cinerea* spores has been reported to be unaffected by this insecticide (Fermaud *et al.* 1994); (iii) Bees were anaesthetised with CO_2 gas while they foraged and were then transferred into vials. (iv) A 25 ml glass Universal bottle was held closely underneath a bee foraging on a kiwifruit flower (which have a pendulous habit), so that on take-off the bee flew into the vial, after which the lid was immediately screwed down firmly. Each bottle contained 5 ml sterile distilled water + 0.05% aqueous 'Tween' 80 surfactant (polyoxyethylene (20) sorbitan mono-oleat). Samples were placed directly into a polystyrene container containing ice-packs and later transferred to a 0°C cool store pending further processing.

2.2.2 RESULTS

Sweep netting and trapping bees on hive landing boards were dismissed due to the following problems: The rapid flight of bees entering the hive prevented most capture efforts; Distinguishing between in-going and out-going bees was difficult; Separating bees foraging on different flower species was impossible; There was a potential loss of propagules during transfer from net to vial; Potential for transfer of propagules between bees via the net; Problems caused by guard bees responding to the researcher's presence close to the hive.

The aerosol insecticide and $C0_2$ anaethatisation methods were also impractical. Spraying bees with insecticide left them wet, sticky, and difficult to handle in relation to ensuring that propagules remained attached to the bees. The sprayed bees also took several hours to die. When anaesthetising bees with $C0_2$ gas, the bees quickly regained consciousness in the open air and often escaped. The gas equipment was also unwieldy to manoeuvre in an orchard setting and this method required the work of two people to collect bees.

Seventy-eighty percent of capture attempts were successful using the method of trapping bees in vials directly from the flowers,. The number of bees collected during a time interval was dependent on the total number of bees foraging in vines. Honey bees seemed less wary and therefore easier to catch most actively when foraging between 1000-1300 h and while vines were at full bloom.

Transferring honey bees directly from flowers into bottles had the following advantages: (i) The technique was simple and easily managed by one person; (ii) The foraging source was guaranteed; (iii) Bees died within 30s of capture when swirled in the surfactant solution, which was considerably faster than death by insecticide, or suffocation in a dry vial; (iv) One or more bees could be collected in one vial by adjusting the amount of liquid in each vial; (v) The risk of propagule loss from samples through handling was minimal because bees were kept in the same container they were collected in through the *B. cinerea* detection process. Samples were processed within 12 h of collection, but some loss of *B. cinerea* spore viability in water may have occurred through leaching (Henis *et al.* 1987).

cinerea spores. Spore germination was investigated in the presence of both pistillate and staminate bee-collected kiwifruit pollen in sterile distilled water (Figure 2-1). Both the percentage germination and the rate of growth of germ tubes was increased in the presence of pollen compared with sterile water alone. This indicated that exudates from pollen grains and honey (regurgitated by bees to assist with transferal of pollen to their corbiculae), would not reduce the viability of spores being carried by bees.

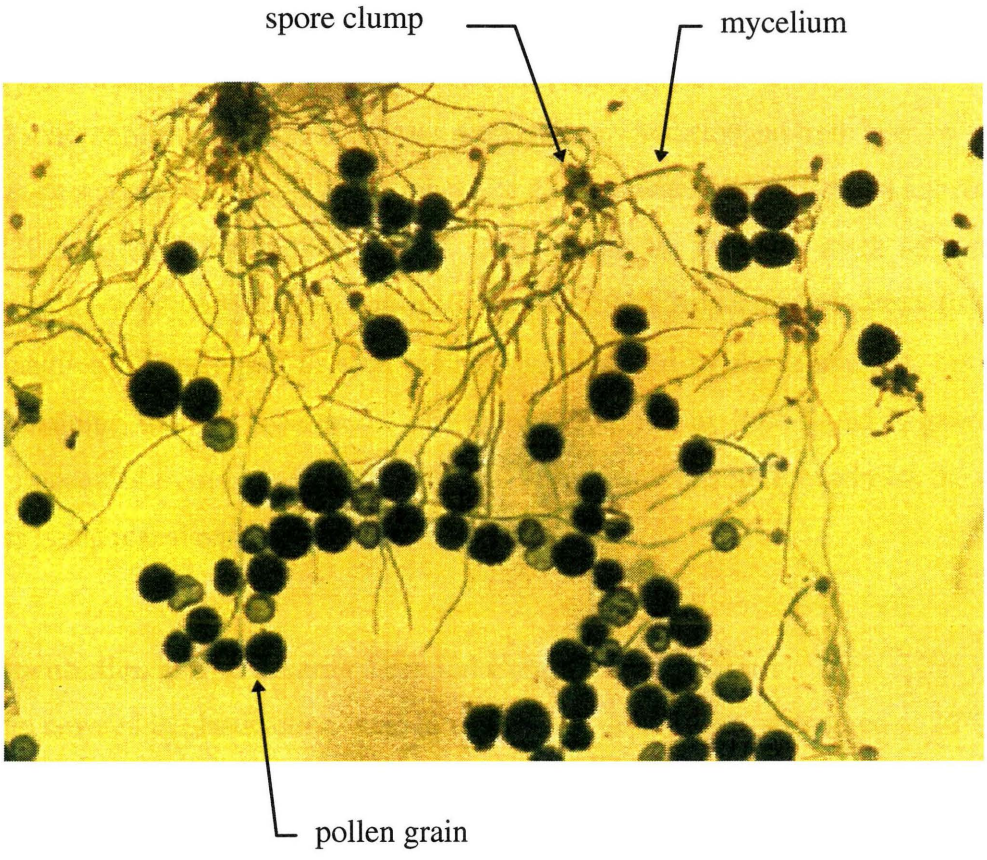


Figure 2-1: *B. cinerea* spores that germinated in the presence of staminate kiwifruit pollen collected naturally by honey bees.

2.3.1 METHODS

Study Sites

Foraging honey bees were collected from two mature, commercial kiwifruit orchards cv. Hayward in the Riwaka area in December 1992. Orchard One (HortResearch) was the same

2.3.1 METHODS

Study Sites

Foraging honey bees were collected from two mature, commercial kiwifruit orchards cv. Hayward in the Riwaka area in December 1992. Orchard One (HortResearch) was the same orchard described in Section 2.2, and Orchard Two (Drummond) was located 5 km to the west of Orchard One. Bees were collected individually from both staminate and pistillate flowers (sample sizes shown in Table 2-1) and stored at 0°C pending further processing.

Isolation Of Viable *B. cinerea* Propagules From Honey Bees

Within 24 h of sample collection, *B. cinerea* propagules were dislodged from bees by vigorously agitating each sample (one bee plus 1.5 ml sterile distilled water + 0.05% (v/v) aqueous Tween 80) for 120 s using a Griffin flask shaker. A 1 ml aliquot from the stock suspension was pipetted onto Kerrsies *Botrytis*-selective agar (Kerssies 1990; Appendix I) in Petri dishes. Most of the remaining 0.5 ml liquid in each sample was absorbed by the honey bee, but the exact amount remaining was not measured. The reason for using as little liquid as practicable to dislodge propagules from bees was to minimise dilution and therefore maximise the chance of propagules being plated onto agar.

Sample Incubation and *B. cinerea* Mycelial Colony Identification

Plates were covered in plastic cling wrap to reduce agar desiccation, and stored at 20°C for 15 h to enhance spore germination. The plates were then incubated for 15-20 days at 10°C without light. The incidence and number of mycelial colony forming units (cfu) of *B. cinerea* were assessed visually by characteristic colour, growth and staining of the surrounding agar to dark-brown (Kerssies 1990). Plates were then incubated for a further 10-14 days at room temperature (18-24°C) under natural light conditions for verification of *B. cinerea* colonies by sporulation characteristics (Coley-Smith *et al.* 1980).

A second method of *B. cinerea* detection was also tested whereby the legs, head and thorax of ten honey bees were placed directly onto plates of *Botryits* selective agar, which were then incubated as above.

Results were expressed as the binary incidence of *B. cinerea* colony forming units per bee.

2.3.2 RESULTS

One hundred and seventy foraging honey bees were collected individually from kiwifruit flowers on two days at late bloom. On 8 December 1992, 61 honey bees were collected from staminate flowers and 61 from pistillate flowers in Orchard One. On 10 December 1992, 48 bees were collected from staminate flowers in Orchard Two (pistillate flowering had ended).

The data on the incidence of natural *B. cinerea* contamination of honey bees are presented in Table 2-1. Note that in Orchard One, there was an equal incidence of *B. cinerea* on honey bees foraging both staminate and pistillate kiwifruit flowers.

Contamination of the *Botrytis*-selective agar medium by *Penicillium* spp. and *Trichoderma* spp. was observed, but the degree to which *B. cinerea* colonies were inhibited or masked was not determined.

Table 2-1: Percent incidence of colony forming units of *B. cinerea* per honey bee foraging on staminate and pistillate kiwifruit flowers in Riwaka at late-bloom in 1992.

	Orchard 1 pistillate 8 Dec 1992	Orchard 1 staminate 8 Dec 1992	Orchard 2 staminate 10 Dec 1992	Overall % Incidence
%	88.5	88.5	83.3	87.1
N	61	61	48	170

The null hypothesis was rejected, as the results supported the alternative hypothesis that viable *B. cinerea* propagules were carried by honey bees foraging on kiwifruit flowers in two orchards in the Riwaka area. *B. cinerea* propagules were present on 87.1% of honey bees.

Number Of Mycelial Colonies

The number of *B. cinerea* colonies per agar plate was counted to estimate cfu loads carried by bees. Although this was not an initial aim of the experiment, it was decided that the information would be useful to record for subsequent experiments. Cfu per bee was calculated by

multiplying cfu per plate by 1.5 to take into account the 1 ml aliquot subsample from 1.5 ml total suspension (Table 2-2). 137/170 (80.6%) bees carried between one and 21 detectable *B. cinerea* cfu. On 11/170 (6.5%) of agar plates, however, the number of individual colonies could not be counted accurately because they had overlapped before the first assessment. These plates probably had 15 or more original colonies because the plates without overlapping colonies all had less than 15 colonies. 148/170 (87.1%) of all bees sampled carried at least one cfu.

Table 2-2: Variability in the number of *B. cinerea* colony forming units (cfu) per agar plate and cfu per honey bee.

Cfu per agar plate	Cfu per bee	N
0	0	22
1 - 5	1.5 - 7.5	129
6 - 14	9 - 21	8
colonies overgrown	>21	11
	Total	**170**

The method of plating dissected honey bees was also found to be impractical due to the high levels of external contamination of bees by other microorganisms, the time required to dissect each bee and difficulties in contacting all of the bees' surface with the agar.

2.3.3 Discussion

The alternative hypothesis that honey bees carry *B. cinerea* while foraging kiwifruit flowers was supported. The high incidence (87%) of viable *B. cinerea* cfu on honey bees foraging on kiwifruit flowers demonstrated their potential as a vector of the fungus. The spread of infection to flowers could contribute to an overall increased inoculum availability throughout the growing season, through the establishment of secondary (and tertiary) foci of infection, and thus potentially lead to an increased risk of picking wound infection at harvest. The foraging activity of honey bees could, therefore, be of significant importance in the epidemiology of *B. cinerea* within kiwifruit orchards (and possibly in the transfer of inoculum between orchards within the foraging range of bees).

The 6.5% of bees that carried enough *B. cinerea* cfu to cover agar plates before the first assessment, may have visited an inoculum source (e.g., a flower or leaf with sporulating *B. cinerea*) shortly prior to collection. These bees may be more likely to transfer inoculum to non-contaminated flowers than bees carrying low numbers of viable propagules.

These results can be compared with estimates of the natural incidence of *B. cinerea* propagules on New Zealand flower thrips (*Thrips obscuratus*) collected from kiwifruit flowers in the Riwaka area by Fermaud & Gaunt (1994). At early flowering in orchards that were heavily infested (10-20 thrips per staminate flower), there was 0-1% incidence of *B. cinerea* on thrips collected from staminate flowers and 0% incidence on thrips collected from pistillate flowers. *B. cinerea* incidence increased to 10-17% at mid-flowering and was 1-10% at petal-fall. The incidence of *B. cinerea* on thrips was, therefore, low compared with that found on honey bees in this study. The movement of thrips is largely determined by wind and is, therefore, more random than honey bee movement (Pyke pers. comm.). Compared with thrips, honey bees may be more likely to come into contact with *B. cinerea* inoculum because individual bees may visit up to 100 flowers per foraging hour (Jay & Jay 1984).

Other studies where the natural incidence of plant pathogens on insects was measured include Fermaud & Le Menn (1989), who isolated *B. cinerea* from 23-99% of samples of grape berry moth larvae on grapes, Huang *et al.* (1986) who *detected Verticillium albo-atrum* (alfalfa wilt) from 30% of leafcutter bees (*Megachile rotundata*), and Jennersten (1983), who found that 50% of all insects that visited caryophyllaceous flowers carried *Ustilago violacea* (anther smut) spores. These findings demonstrate that large insects other than honey bees are able to acquire pathogen inoculum from the plants they visit.

In this experiment control samples should have been taken to determine the number of *B. cinerea* propagules which were inadvertantly collected while unattached to bees (e.g., from the air or dislodged from flowers during sampling). This problem is addressed in Section 2-3. Two further sources of error which were not estimated were the percentage loss in propagule viability between the time of honey bee collection and plating of propagules onto agar, and the proportion of propagules carried by bees that were detected using this method. These factors could be studied in future experiments to enable more accurate estimation of the incidence and number of

propagules carried by individual honey bees. The accuracy of the detection method could be estimated by inoculating 'clean' bees (e.g., surface sterilised bees) with a known number of viable *B. cinerea* spores, then using the detection technique to determine the efficiency of removal. A qualitative test would be to use scanning electron microscopy to look at the hairs of inoculated and washed bees for *B. cinerea* spores.

Agar Contamination

Kerssies *Botrytis*-selective medium was prone to contamination by a number of microorganisms. An agar medium with better selectivity for *B. cinerea* would increase the probability of detecting *B. cinerea* cfu by reducing competition with other microorganisms. A recently developed *Sclerotinia sclerotiorum*-selective medium has excellent potential to be adjusted to select for *B. cinerea* (Knight & Bourdot in prep.). Low pH is the main selective mechanism of the medium (pH 3.10-3.25 c.f. pH 4.5 Kerssies medium), so that highly toxic chemicals are not required for selectivity. *B. cinerea* grows and sporulates well on agar at this pH, while commonly occurring fungi such as *Penicillium* spp., *Mucor* spp., *Fusarium* spp. and *Trichoderma* spp. are excluded.

2.4 VARIATION IN NUMBER OF *B. CINEREA* CFU CARRIED BY HONEY BEES AT EARLY, MID AND LATE KIWIFRUIT FLOWERING

The aim of this section was to quantify the viable *B. cinerea* propagules carried by honey bees. The first H_0 was that the mean number of *B. cinerea* cfu carried by honey bees did not vary between early, full and late kiwifruit bloom. The second H_0 was that the average cfu load did not vary between honey bees foraging on three kinds of flowers (flower 1, 2, 3).

2.4.1 METHODS

This study was conducted in December 1993 in a mature kiwifruit orchard (HortResearch) described in Section 2.2.1.

Collection Of Honey Bees

The number of *B. cinerea* cfu carried externally by honey bees foraging on staminate and pistillate kiwifruit flowers was compared with the number carried by honey bees foraging on manuka. Manuka was selected because foraging activity was high on these plants and they were within foraging range (c.500 m) of the HortResearch kiwifruit orchard.

Honey bees were collected on three days during kiwifruit flowering to coincide with 'early' (10-15%) pistillate bloom (4 December 1993), 'full' (70%) bloom (10 December 1994) and 'late' bloom (most flowers senescing) (15 December 1993). Twenty samples, each comprising 10 honey bees collected in a Universal bottle containing 6 ml sterile distilled water plus 0.05% Tween 80 (v/v), were collected from each flower type on each of the three days. Samples were stored in a polystyrene box with ice-packs and later transferred to a 0°C cool store for 4-12 hours pending further processing.

Control Samples

The number of *B. cinerea* propagules that were collected with honey bees but which may have come from the air or been dislodged from flowers by bees (i.e., not actually carried by bees) was assessed by holding a universal bottle containing 6 ml sterile distilled water plus 0.05% Tween 80 (v/v) beneath a foraging bee, then removing the bottle quickly as the bee took off so that it was not trapped. Ten control samples, each held under ten bees, were collected from each flower type on each day.

Isolation Of Viable *B. cinerea* Spores From Honey Bees

The method of detecting *B. cinerea* cfu from bees was adjusted slightly from that described in Section 2.2.2 so that, depending on treatment type, both undiluted and diluted propagule suspensions were plated onto agar to ensure accurate colony counts were obtained. The following dilution series was made for each sample excluding controls: 1:1, 1:10 and 1:100. Control samples were not diluted because low numbers of cfu *B. cinerea* and few contaminants were expected. Seven hundred and fifty µl aliquots from each dilution were pipetted onto Botrytis-selective medium (Appendix I) in Petri dishes and spread evenly with a sterile glass instrument shaped like a hockey stick. Plates were incubated and mycelial *B.*

cinerea colonies were counted as described in Section 2.2.1. Results were expressed as the mean number of *B. cinerea* cfu per bee or per control sample.

Statistical Analysis

The control treatment was not included in the main analysis. The dependent variable *B. cinerea* cfu was square root transformed and data were analysed using a 3 x 3 (times x flower types) factorial analysis of variance. Linear and quadratic components were specified for the time factor and two orthogonal contrasts (kiwifruit flowers versus manuka flowers, and staminate flowers versus pistillate flowers) were specified for the flower type factor.

2.4.2 RESULTS

Staminate vines were in full bloom at 40-50% pistillate bloom. During early and late pistillate and staminate kiwifruit flowering, fewer flowers were available for foraging, therefore it took several hours longer to find and collect bees than during full bloom.

The numbers of cfu detected in control samples were negligible, so all cfu detected in bee samples were assumed to have come from honey bees. One datum point (320 cfu per bee at mid flowering on staminate flowers) was regarded as an outlier after plotting a graph of the residuals versus fitted values. This datum point was replaced with a missing value generated by a Genstat procedure. The analysis of variance is presented in Table 2-3. The average numbers of *B. cinerea* cfu detected per honey bee are presented in Table 2-4 and Figure 2-1. The significance of interaction effects are presented in Table 2-5. Raw data are presented in Appendix II.

Table 2-3: Analysis of variance of linear and quadratic components for time (early, full and late pistillate kiwifruit flowering) and two orthogonal contrasts specified for flower type factors (data √ transformed). Sums of squares (SS), degrees of freedom (df), F values (F), F probability (P).

SOURCE OF VARIATION	SS	DF	MS	F	P
Time					
Linear component	113.514	1	113.514	57.61	<.001
Quadratic component	5.849	1	5.849	2.97	0.087
Flower					
Kiwifruit vs. Manuka	10.577	1	5.849	5.37	0.022
Staminate vs. Pistillate	2.733	1	2.733	1.39	0.241
Flower vs. Time					
Flower (kiwifruit vs. manuka) vs. Time (linear component)					
	10.327	1	10.327	5.24	0.023
Flower (kiwifruit vs. manuka) vs. Time (quadratic component)					
	4.549	1	4.549	2.31	0.131
Flower (staminate vs. pistillate) vs. Time (linear component)					
	2.136	1	2.136	1.08	0.299
Flower (staminate vs. pistillate) vs. Time (quadratic component)					
	0.029	1	0.029	0.01	0.904
Residual	334.966	170(1)	1.970		
Total	484.506	178 (1)			

Table 2-4: Mean number of *B. cinerea* colony forming units per honey bee and per control sample (data √-transformed; back-transformed values in parentheses).

	Stage of Kiwifruit Flowering					
	Early flower (4 Dec 1993)		Full flower (10 Dec 1993)		Late flower (15 Dec 1993)	
Flower Type	**Bee**	**Control**	**Bee**	**Control**	**Bee**	**Control**
Pistillate kiwifruit	1.38 (1.90)	(0)	2.99 (8.94)	(0)	3.42 (11.70)	(0.1)
Staminate kiwifruit	1.33 (1.77)	(0)	3.33 (11.09)	(0.1)	4.02 (16.16)	(0)
Manuka	2.73 (7.45)	(0.1)	3.20 (10.24)	(0)	3.85 (14.82)	(0)

LSD (5%) for comparing main effect means (both flower type and flowering stage) = 0.51

Table 2-5: Significance of interactions.

Source of Variation	
Flower (kiwifruit vs. manuka) vs. Time (linear component)	*
Flower (kiwifruit vs. manuka) vs Time (quadratic component)	n.s.
Flower (staminate vs. pistillate) vs. Time (linear component)	n.s.
Flower (staminate vs. pistillate) vs. Time (quadratic component)	n.s.

* = significant at the 5% level

Time

There was a highly significant ($P < 0.001$) linear trend in the number of *B. cinerea* cfu carried by bees over time. The quadratic component over time was significant at the 10% level ($P = 0.087$) describing the slight flattening of this curve between full and late bloom.

Flower Type

The number of *B. cinerea* cfu detected on manuka-foraging bees was significantly higher ($P = 0.022$) than the number carried by kiwifruit-foraging bees ($P < 0.05$). This occured mainly at the 'early flower' period. The least significant difference (LSD) (5%) (comparing main effect means) shows significantly higher cfu loads on bees foraging staminate flowers at late flower.

Flower Type x Time

The contrast between kiwifruit and manuka flowers had a significant linear component at the 5% level ($P=0.023$, i.e., the relationship varied over time). A steeper increase in the number of *B. cinerea* cfu carried by kiwifruit-foraging bees was found between early and mid flowering, compared with manuka-foraging bees. There were no significant linear or orthogonal components to the interaction between bees foraging staminate versus pistillate flowers over time (i.e., the number of cfu increased at a constant rate over time).

Data Outlier

By including the datum value which was excluded as an outlier, the average number cfu detected per bee foraging staminate flowers at full bloom was more than doubled from 11.79 to 27.2 (data back-transformed). The quadratic component of time became significant at the 5% level ($P=0.027$). Staminate versus pistillate became significant at the 10% level

(P=0.094), and the linear component of staminate versus pistillate over time became significant at the 10% level (P=0.071).

2.4.3 Discussion

The hypothesis that the average number of *B. cinerea* cfu carried by honey bees increases from early flowering through to late kiwifruit flowering was supported. Elmer *et al.* (1992) showed that staminate flowers were a major source of *B. cinerea* inoculum in kiwifruit vines at early and full kiwifruit flower, while pistillate flowers become a major inoculum source closer to petal fall. This is probably attributable to the fact that staminate flowering begins earlier than pistillate, thus senescing earlier, and necrotic staminate flower tissues are available for colonization first. Elmer *et al.* (1992) also found that average numbers of airborne *B. cinerea* cfu increased from before full kiwifruit bloom to one week after petal fall. The numbers of cfu sampled varied widely from day to day over the kiwifruit flowering period (e.g., c.10-170 cfu on consecutive days). Similarly, the number of *B. cinerea* propagules carried by honey bees could vary between days depending on environmental variables that affect spore production. On average, Elmer *et al.* (1992) sampled c.20 *B. cinerea* cfu per 12 hr sample period (5.4×10^5 L air) during flowering. This corresponds to one *B. cinerea* cfu per 2.7×10^4 L air. Compared with airborne spores, honey bees could be vastly more important in the epidemiology of *B. cinerea* by carrying 2-19 cfu and potentially vectoring them directly to host tissue.

The result that *B. cinerea* was carried by honey bees visiting manuka flowers within foraging range of kiwifruit orchards, demonstrated that they could present a source of *B. cinerea* inoculum for kiwifruit flower infection. Viable *B. cinerea* propagules may be transferred between bees as they brush against each other inside the hive, as with pollen (Free & Williams 1972). In this way, inoculum picked up by bees foraging on one flower type could be transferred to bees foraging on other flower types. Alternatively, a bee that had picked up *B. cinerea* inoculum while collecting nectar or pollen from an alternate flower source to kiwifruit, could switch to collecting pollen from kiwifruit flowers on a consecutive trip, depending on hive food requirements. In a highly intensive horticultural area such as Riwaka, bees from any one hive would be within foraging range of a variety of flowering crops, pastures and home gardens. *B. cinerea* has a wide host range, and many species of flowering host plants are be in bloom at

the same time as kiwifruit (e.g., berryfruit, beans, tomato, flax and clover). The number of honey bees from hives placed in kiwifruit orchards that forage flowers other than kiwifruit, is mainly due to their colonies' requirement for nectar, which kiwifruit does not supply. Providing sugar syrup inside hives to reduce numbers of bees foraging for nectar, may be a useful 'cultural' control strategy to reduce the amount of *B. cinerea* inoculum introduced from outside the orchard by vector bees foraging on non-kiwifruit flowers (e.g., manuka) in search of nectar.

The steeper increase in the number of cfu per kiwifruit-foraging bee between early and mid flowering, compared with manuka-foraging bees, may be because manuka blooms over a longer time period, so that the change in the amount of necrotic flower tissues available for *B. cinerea* infection and sporulation is not as great as with kiwifruit.

Outlier

The datum value of 320 cfu per bee, viewed as an outlier in the main analysis, may in fact have been representative of the population. At a rate of eight hives per hectare, there would be a maximum of 160 000 bees foraging per ha of orchard. Extrapolating even further, if a small percentage of bees (e.g., 0.05%) carried a high number of propagules (e.g., >100 cfu per bee), this would equate to 80 bees per hectare, each foraging up to 100 flowers per hour.

Future Work

The experimental design of this study could be improved by including a random sampling plan. Vines or areas of vines could be allocated to experimental blocks prior to sampling, taking into account possible edge effects on foraging behaviour due to shading by shelter. The sampling design would also need to take into account the ratio and distribution of pistillate to staminate plants. Data from Sections 2.3 and 2.4 showed no significant difference between the number of *B. cinerea* propagules being carried by bees foraging on staminate and pistillate flowers, so bees could be collected regardless of what sex of flower they forage. If the ratio of staminate:pistillate vines was, for example, 1:6, then 1/6 of honey bees collected in a sample could be collected from staminate flowers.

2.5 CHAPTER SUMMARY

- The result that honey bees carried *B. cinerea* externally in Riwaka kiwifruit orchards was a first step in defining the role of this insect in the dissemination of *B. cinerea* inoculum to kiwifruit flowers and also in the etiology of stem-end rot of kiwifruit. The presence of *B. cinerea* cfu on approximately 87% of honey bees sampled individually while foraging on kiwifruit flowers illustrated their potential role in transferring the pathogen, and possibly other pathogens, to kiwifruit flowers.
- The mean number of *B. cinerea* cfu carried by kiwifruit-foraging honey bees increased from early to late stages of flowering.
- The mean number of *B. cinerea* cfu carried by kiwifruit-foraging honey bees ranged from zero to 320 cfu per bee.
- *B. cinerea* cfu were detected on honey bees visiting manuka flowers within foraging range (c. 500 m) of several kiwifruit orchards.
- Facets of the experimental designs that could be improved were discussed. Such improvements could increase the accuracy of estimates and also allow a closer examination of this vector system over time.

3. ACQUISITION OF *B. CINEREA* SPORES FROM KIWIFRUIT FLOWERS BY HONEY BEES

3.1 INTRODUCTION

B. cinerea conidia are liberated passively by the action of external energy (Gregory 1973), and wind is thought to be the primary liberating force (Aylor 1990; Henis *et al.* 1987). *B. cinerea* conidia may also be liberated by contact with insects. The acquisition of *B. cinerea* conidia from kiwifruit flowers by the New Zealand flower thrips has been demonstrated (Fermaud & Gaunt 1995; Fermaud *et al.* 1994). In Chapter Two, it was established that up to 87% of honey bees carry viable *B. cinerea* propagules externally while foraging on kiwifruit flowers. These bees may have picked up conidia from infected kiwifruit flowers.

In this Chapter, an experiment is described that aimed to determine whether or not honey bees picked up *B. cinerea* propagules while they foraged on inoculated kiwifruit flowers. H_0 was that honey bees foraging on kiwifruit flowers inoculated with *B. cinerea* conidia carry more detectable *B. cinerea* colony forming units on their bodies than bees collected after foraging on non-inoculated flowers.

3.2 METHODS

Study Site

This experiment was conducted in December 1993 in a mature kiwifruit orchard at Riwaka described in Section 2.2. Two neighbouring (across rows) staminate vines were selected which were within 40 m of two active honey bee hives. No natural *B. cinerea* sporulation was observed on flowers in the experimental vines. The vines that were selected were nearing full bloom and being visited more regularly by honey bees compared with other vines in the orchard which were only in the early stages of flowering (approximately 20% bloom). The use of staminate flowers had the advantage over pistillate flowers that they hang in bunches, so that a greater number of flowers could be observed for honey bee visits. Two 1.0 m^2 'quadrats' of canopy containing 30-40 open staminate flowers were marked out with string in each of the experimental vines.

Honey Bee Collection

Honey bees were collected while foraging on kiwifruit flowers that had either been treated (inoculated with *B. cinerea* spores) or not treated. On 6 December 1993, flowers in the quadrats were observed and honey bees collected as they completed foraging uninoculated flowers to determine the number of cfu *B. cinerea* they carried naturally. Bees were trapped individually in Universal bottles containing 2 ml sterile distilled water plus 0.05% aqueous Tween80 (v/v) as described in Section 2.2. On 7 December 1993, flowers in the quadrats were inoculated with *B. cinerea* spores (method described below). The flowers were then observed and honey bees collected as they completed foraging the inoculated flowers. Fifty five bees were collected from uninoculated flowers, and another 55 from inoculated flowers. Bees were transferred to 0°C cool storage pending further processing.

Control Samples

Separate 'Universal' bottles containing 2 ml sterile distilled water plus 0.05% Tween80 (v/v) were held under individual foraging bees without trapping them to measure the number of *B. cinerea* propagules collected from the air or that were dislodged from flowers. Each bottle was held under one foraging bee. Twenty samples were collected from uninoculated flowers, and 20 from inoculated flowers and transferred to 0°C cool storage pending further processing.

Flower Inoculation Technique

The aim of flower inoculation was to have conidia available on anthers and petals so that honey bees would come in contact with them during normal foraging movement. A high number of spores on flowers was required to increase the likelihood of detecting spore pick up by bees. Sporulating cultures of *B. cinerea* were produced on V8-juice agar (Appendix I) in Petri dishes after 10-13 days incubation at 20°C with a 12 h photoperiod of fluorescent daylight and near-UV light. Several methods of flower inoculation were tested as follows: (i) Flowers were sprayed with *B. cinerea* spore suspensions to cause natural infection and sporulation. Unfortunately the vines were accidentally sprayed with fungicide by an orchard worker before sporulation was observed. Due to time constraints, it was then decided to concentrate on 'artificially' contaminating flowers to mimic natural sporulation; (ii) Small squares of agar carrying sporulating cultures were stuck to petals, but the agar pieces did not adhere well and were too

small and flexible to attach to petals with many spores intact; (iii) Dry spores were applied onto anthers and petals with a paint brush, but the brush became matted with spores and mycelium which made spore transfer onto flowers difficult and spore coverage was insufficient; (iv) Cultures of sporulating *B. cinerea* in Petri were pushed directly against kiwifruit flowers so that the anthers and petals were inoculated with high numbers of dry spores, similar to natural sporulation. Spores were observed as a grey covering on the inoculated flowers. Method (iv) was the most successful and was that used to conduct the experiment.

B. cinerea Detection

The *B. cinerea* propagules were rinsed from bees by vigorously agitating each sample for 120 s using a mechanical shaker. Control samples were treated in the same way. High numbers of *B. cinerea* cfu were expected from samples of honey bees collected from inoculated flowers. Stock suspensions from these samples were diluted to concentrations of 1:1 (stock suspension), 1:10 and 1:100. Bees from uninoculated flowers, and control samples from inoculated flowers, were diluted to give concentrations of 1:1 and 1:10. Low cfu numbers were expected from control samples from uninoculated flowers, so the stock suspension was not diluted. One 750 µl aliquot from each dilution was spread on *Botrytis*-selective agar (Appendix II). Plates were incubated and mycelial *B. cinerea* colonies counted as described in Section 2.2.1. Results were expressed as the average number of *B. cinerea* cfu per honey bee, or per control sample.

Statistical Analysis

There were large differences in data between treatments and so analysis of variance was not required to test for significance. Data were square root transformed and treatments compared by means and 95% confidence intervals.

3.3 RESULTS

Raw data are presented in Appendix II. The sample sizes were determined by the number of bees foraging and the number of successful captures. Foraging activity per quadrat decreased with increasing number of bee captures. The number of foraging trips each bee had made to inoculated flowers before capture was not known because only one quadrat was observed at a time and some capture attempts were unsuccessful. The data are summarised in Table 3-1.

The number of propagules picked up by honey bees from inoculated flowers was significantly higher than that carried by bees foraging on uninoculated flowers. The number of propagules picked up by honey bees was significantly higher than the number collected in control samples (i.e., dislodged from flowers by bees during foraging, or present as background levels in the air). This demonstrated that at least 95% of the *B. cinerea* cfu detected were attached to honey bees.

Table 3-1: Square root of the mean number of *B. cinerea* colony forming units per sample for treatments ± collection of bees (bees) and ± inoculation of flowers with *B. cinerea* spores (inoculation). 95% confidence intervals and back-transformed data (mean number of cfu per sample).

Treatment	Mean	95% CI	Mean (backtransformed)
+ Bee, + Inoculation	63.2	± 6.1	3994
+ Bee, - Inoculation	2.6	± 1.3	6.8
- Bee, + Inoculation	3.7	± 2.2	13.7
- Bee, + Inoculation	0	± 0	0

3.4 DISCUSSION

The hypothesis that honey bees foraging on kiwifruit flowers inoculated with *B. cinerea* conidia carry more detectable *B. cinerea* cfu on their bodies than bees collected after foraging on non-inoculated flowers was supported. High numbers of *B. cinerea* propagules (mean 3994 cfu) were acquired by bees foraging on inoculated flowers compared to uninoculated flowers (mean 6.8 cfu). One thousand one hundred and twenty *B. cinerea* cfu were detected from one honey bee visiting an uninoculated flower (Appendix II). This bee may have visited a flower with a natural sporulating infection shortly prior to capture.

Flowers were not collected to determine their spore-load in this experiment. Similarly-inoculated flowers collected in a subsequent experiment (Chapter Five) carried an average of 6.2 x 10^4 *B. cinerea* cfu each. It would be useful in a future study to inoculate flowers with known numbers of dry spores, and determine the minimum and maximum numbers of *B. cinerea*

propagules that honey bees can pick up during one flower visit. This could then be related to spore transfer to healthy flowers and the likelihood of infection.

Researchers have also demonstrated the acquisition of *Erwinia amylovora* (Johnson *et al.* 1993), *Pseudomonas fluorescens* (Johnson *et al.* 1993; Thomson *et al.* 1992) *E. herbicola*, (Thomson *et al.* 1992) and *Gliocladium roseum* (Peng *et al.* 1992) propagules by honey bees using special inoculum dispensers at bee hive exits. Using this inoculation method, bees that were forced to crawl through inoculum while exiting the hive acquired up to 8.9×10^5 cfu *G. roseum* from crawling in preparations containing 1×10^8 cfu *G. roseum* per g (Peng *et al.* 1992), and an average of 1×10^5 *P. fluorescens* and 1×10^4 *E. herbicola* cfu per bee (Thomson *et al.* 1992).

Accumulation Of Inoculum On Honey Bees

The way in which *B. cinerea* conidia adhere to honey bees may impact on the probability of conidia being transferred to healthy flowers. Most pollen transferred from a foraging honey bee to the stigma of a kiwifruit flower comes from the forager's body rather than from the pollen already packed into its corbiculae (Goodwin & Perry 1992). Conidia may also be packed into the corbiculae and be unavailable for transfer. Areas on a bee where conidia may remain ungroomed and therefore more readily available for transfer include the top and middle thoracic segment which constitutes a 'blind spot' where bees cannot groom pollen with their middle legs (Winston 1987). Also the back of the head, the central dorsal part of the first thoracic segment and the first and second abdominal terga are infrequently groomed (Free & Williams 1972).

B. cinerea conidial adhesion to honey bees could be examined in future work by examining bees that had foraged on inoculated flowers using a microscope. With a binocular microscope, Peng *et al.* (1992) observed *Gliocladium roseum* inoculum on almost all external surfaces of honey bees that had crawled in infested substrate. Conidia were most dense near the bases of the femoral setae, which presented a large surface area to which inoculum could adhere to. Huang *et al.* (1986) examined female leafcutter bees (*Megachile rotundata*) under a scanning electron microscope and detected *Verticillium albo-atrum* conidia most frequently on the mouth parts, abdomen, and legs.

The surface electric potentials of honey bees may also effect their ability to pick up *B. cinerea* conidia while foraging. Erickson (1975) found that honey bees entering and leaving the hive had surface electric potentials that followed a daily rhythm, with a peak at midday or in the early afternoon. Bees entering the hive were normally positively charged, while bees leaving the hive had a more uniform potential, normally slightly negative early in the day, becoming slightly positive as the day advanced. The magnitude of all potentials was dependent in part on weather conditions. It would be useful to determine the electric charge on *B. cinerea* conidia and whether or not this effects spore adhesion to bees.

The presence of pollen as a nutrient source may also increase the germination rate and infection ability of conidia once deposited on flower parts by honey bees. This hypothesis is supported by the result that *B. cinerea* spore germination rate and mycelial growth were increased in the presence of kiwifruit pollen in sterile distilled water (Section 2.3). Hartnill (1975) demonstrated enhanced *B. cinerea* spore germination and mycelial growth on tobacco (*Nicotiana tabacum*) leaves in the presence of tobacco pollen, and Chou & Preece (1968) demonstrated stimulation of *B. cinerea* spore germination, mycelial growth and lesion development on strawberry (*Fragaria ananassa*) petals and broad bean (*Vica faba*) leaves in the presence of strawberry and bean pollen.

3.5 CHAPTER SUMMARY

The results of this study demonstrated that honey bees are capable of collecting large numbers of dry *B. cinerea* spores while foraging on kiwifruit flowers artificially contaminated with spores, providing evidence that they may also collect spores from naturally-infested flowers.

4. TRANSFER OF *B. CINEREA* BETWEEN KIWIFRUIT FLOWERS BY HONEY BEES

4.1 INTRODUCTION

The severity of diseases caused by aerially-dispersed plant pathogens generally decreases rapidly with increasing distance from a focus of infection. Empirical and physical models have been developed to describe the relationship between the numbers of spores deposited on foliage and other surfaces, downwind from a release point. These models are important in aids to understanding the rate of spatial spread of epidemics, and can be incorporated into epidemic models as mathematical descriptions of spore dispersal gradients, dy/dx, where y is the number of spores deposited per unit of leaf area and x is distance downwind from the source (Aylor 1990). Deposition gradients modelled empirically are often found to follow either a negative exponential law [$y=A\ exp(-Bx)$] or an inverse power law [$y=a\ x^{-b}$] (Aylor 1978; Gregory 1973; Fitt & McCartney 1986; Ward *et al.* 1989).

Physical models can also be used to describe spore dispersal (Fitt & McCartney 1986). Three such physical models, which were initially constructed to describe gas or aerosol pollution dispersal, have also been applied to spore dispersal: the 'Gaussian Plume' model (Aylor 1978; De Jong *et al.* 1990) uses a statistical approach, the gradient diffusion theory (Aylor 1990) uses a diffusion approach, and random walk models (Fitt & McCartney 1986) use a stochastic approach.

The aerial dispersal of some insect vectors of plant and animal diseases, such as viruliferous aphids, mites (Wiktelius 1980), mosquitoes and stable flies (*Stomoxys calcitrans*) (Pedgely 1982), have been studied in some detail for the purpose of forecasting epidemics, often on a national, or in some cases a continental, scale. In studying honey bee/plant pathogen interactions, pathologists have focused mainly on the dispersal of propagules to commercially-important fruit flowers by bees that have been artificially contaminated at the hive. In this study, the dispersal of inoculum between kiwifruit flowers by honey bees was of interest because flowers infected with *B. cinerea* may be considered as inoculum foci.

Two hypotheses were tested in this experiment. The first H_0 was that the number of *B. cinerea* propagules transferred by honey bees to kiwifruit flowers decreases with distance from a source of flowers contaminated with *B. cinerea* spores. The second H_0 was that kiwifruit flowers visited by honey bees within 2 m of a source of flowers contaminated with *B. cinerea* spores, carry a higher mean number of *B. cinerea* propagules than similarly positioned flowers which are not visited by bees.

4.2 METHODS

This experiment was conducted in December 1993 in a mature kiwifruit orchard in Riwaka described in Section 2.2.1. Six rows of kiwifruit were available for use. The transfer of *B. cinerea* inoculum to pistillate, but not staminate flowers, was studied so that honey bee foraging behaviour could be regarded as uniform with regard to preference for one sex of flower. Staminate vines were planted individually, spaced every three pistillate vines in every second row. It was decided that each staminate vine did not cover a large enough area of continuos canopy to use in this experiment. Inoculum transfer was measured along rows, compared to across rows, to provide 5 m continuous sections of flowering canopy and to take into account the observation that over 60% of honey bees forage along rows (Jay & Jay 1984).

Six replicate plots (A-F) were established in six rows of kiwifruit at pre-flowering (flowers beginning to show white through splitting sepals). Each plot consisted of a 5 m length of continuous pistillate kiwifruit canopy (approximately 1.5 vines per plot). Plots were selected for both high flower numbers and even flower spread. Plots were separated by at least three vines within rows and one vine between rows to minimise interplot interference. All plots and immediate neighbouring vines at each end of plots were sprayed to control New Zealand flower thrips (*Thrips obscuratus*) at 5% pistillate flowering (1 December 1993) with the insecticide fluvalinate (Mavrik 24FLO, 9.6 g active ingredient/100 L). This was a precaution against the possibility that high numbers of thrips could disperse enough *B. cinerea* spores to significantly affect results (Fermaud & Gaunt 1994). Spraying occurred before beehives were placed in the orchard to minimise the impact on honey bees (Johansen *et al.* 1981). Eight hives per ha were placed at the ends of kiwifruit rows at 10% pistillate flowering (3 December 1993).

Plot Inoculation

Each plot was split into three sections (Figure 4-1). At full bloom, dry *B. cinerea* spores were applied to flowers (as described in Section 3.2) within the central 2 m^2 section of canopy (termed an 'inoculum zone') between 0600 h and 0730 h on a day forecast to be sunny when bee activity was probable (12 December 1993). Spores were applied to all flowers (approximately 80 per plot) in the inoculum zones, apart from flowers allocated to the wind dispersal and control treatments (see below). Flower samples in the outer 2 x 4 m^2 sections were collected 8 h after spore application, to examine variation in *B. cinerea* dispersal with distance (0 - 2 m) in both directions along rows from the inoculum zone. Eight double-flower samples of inoculated flowers were collected per plot after 8 h foraging.

Treatments

Samples comprised two kiwifruit flowers <50 mm apart on the vine. Two flowers were used to increase the probability of detecting low numbers of spores per flower. Three treatments were used to distinguish between the following modes of inoculum dispersal: (i) No Dispersal Agent (Control). *B. cinerea* dispersal to flowers by wind and insects was prevented by enclosing samples in greaseproof paper bags (25 x 25 cm) at pre-flowering. A 5 mm diameter hole was cut in the lower corner of each paper bag and plugged with cotton wool to allow rain water to drain from bags in the event of wet weather; (ii) Inoculum Dispersal By Wind. *B. cinerea* dispersal to flowers by honey bees and similar sized insects, was excluded by enclosing flowers in bags made of black plastic shade cloth (2 mm^2 mesh), enabling only dispersal by wind and by insects of diameter < 2 mm; (iii) Inoculum Dispersal By Honey Bees. *B. cinerea* dispersal to flowers by honey bees, wind and other insects was facilitated by leaving flowers un-bagged. The effect of spore transfer by honey bees and other insects could be estimated by comparing the effects of the wind and control treatments.

Sample Size And Treatment Lay-Out

The main aim of this experiment was to measure spore dispersal by honey bees. Use of available flowers was optimised by conducting fewer control and wind treatments than honey bee treatments. Foraging movement of honey bees was assumed to be equal up and down rows, so inoculum dispersal was measured in both directions (North or South, Figure 4-1) along rows

from the central inoculum zone to increase available flower numbers. Wind and control treatments were placed in the inoculum zone and in one direction only per plot. The direction that these treatments were placed was allocated randomly to plots, with three plots per direction (Figure 4-1).

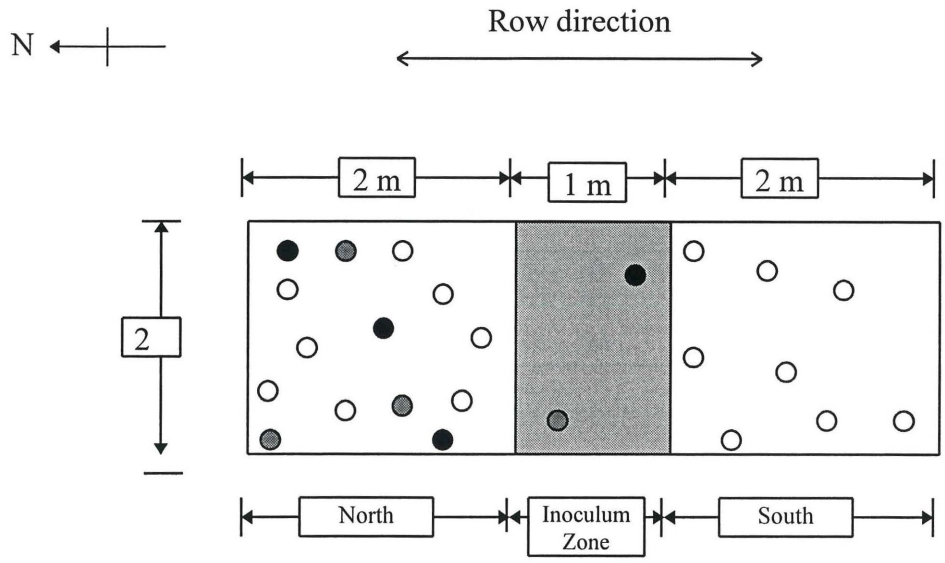
Deposition Gradient

The relationship between the number *B. cinerea* cfu deposited per flower with varying horizontal distance from the inoculum zone was measured by sampling as evenly as possible between 0.1 - 2 m (+/-0.05 m) from the outside edge of each zone (Figure 4-1). All samples within the inoculum zones were labelled as distance '0 m'. The distance between each sample and the inoculum zone was recorded on a tag and kept with the sample through to processing. There were not enough flowers in some plots to have even numbers of samples per treatment and flowers were not distributed uniformly. At some distances therefore, several samples were taken, while at other distances no samples were taken. The number of double-flower units sampled in the inoculum zone and in directions North and South per treatment are presented in Table 4-1.

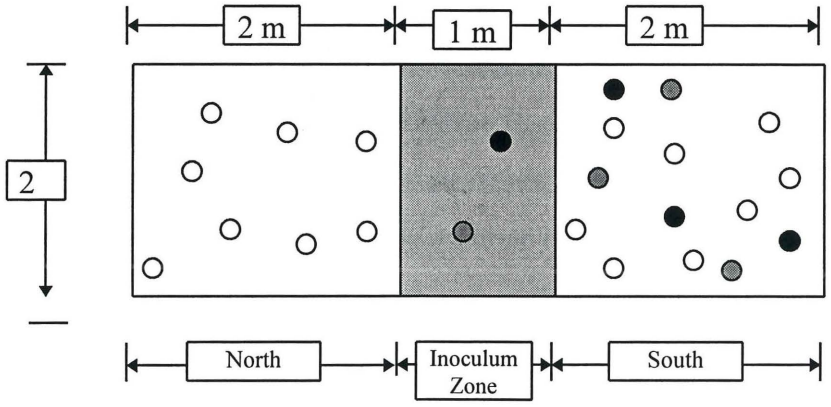
Foraging Activity

Honey bees were observed visiting un-bagged flowers for up to 8 h on the day of spore application. A negligible number (approximately 1% of insect visits to flowers) of bumblebees (*Bombus* spp.), native bees (*Leioproctus* spp.) and hover flies (*Melangyna novaezelandiae* and *Melanostoma fasciatum*) were also observed visiting kiwifruit flowers. Zero to five thrips per flower had reinfested vines by full bloom on the day the experiment was conducted.

Plots A, B and D - Wind and control treatments in northerly direction only:



Plots C, E and F - Wind and control treatments in southerly direction only:



KEY to flower treatments*

- ● Control
- ◍ Wind dispersal
- ○ Honey bee dispersal

***Note:** Each double-flower control, wind and honey bee sample (● ◍ ○) in the diagram corresponds with three field samples.

Figure 4-1: Plot layout, showing allocation of inoculum dispersal treatments (control, wind and honey bee) to double-flower samples in 'north', 'south' and 'inoculum' zones in each plot.

Table 4-1: Number of double-flower samples collected per plot.

PLOTS A, B, D	artificially-contaminated flowers	DISPERSAL AGENT TREATMENT honey bee	wind	control
Inoculum Zone	8	0	3	3
North	0	24	9	8
South	0	24	0	0
PLOTS C, E, F				
Inoculum Zone	8	0	3	3
North	0	24	0	0
South	0	24	9	8

Sample Collection And *B. cinerea* Detection

At 1800 h on the same day as *B. cinerea* application to flowers, when honey bees had become inactive, all flower samples were harvested and stored separately in small plastic bags at 0°C. Samples were processed within seven days of collection. The *B. cinerea* propagules were rinsed from flowers by agitating each sample in 15 ml sterile distilled water + 0.01% Tween80 (v/v) in a 20 ml pomade bottle for 120 s using a Griffin flask shaker. High numbers of cfu were expected on flowers in the honey bee dispersal treatment, so serial dilutions were made of wash-water at 1:1, 1:10, 1:100 and 1:1000 to enable accurate colony counts on agar. Lower numbers of cfu were expected on flowers in the wind and control treatments, so serial dilutions were made at 1:1, 1:10 and 1:100. One 75 µl aliquot from each dilution for each sample was plated onto *Botrytis*-selective agar (Appendix I) in Petri dishes. Plates were incubated and colonies counted as described in Section 2-2. Where the number of *B. cinerea* cfu per plate was less than 200, colony counts were taken from the most concentrated aliquot for each sample. Individual *B. cinerea* colonies could not be accurately counted on some plates at 1:1 dilution because colonies overlapped or were masked by the growth of contaminant microbes such as *Penicillium* spp and *Trichoderma* spp. In some cases, colony counts from higher dilutions were important to refer back to, to help account for occasional unexpected differences in the magnitude of counts at concentrations of 1:1 and 1:10. Colony counts were converted to mean number of detectable cfu *B. cinerea* per flower.

Assumptions For This Statistical Analysis

For the purposes of analysis, all *B. cinerea* propagules detected on flowers in the honey bee dispersal treatment were assumed to have been transferred by honey bees, although a small percentage may have been transferred by insects other than honey bees, or by wind.

All samples located the same distance from an inoculum zone were considered independent samples because they had different positions in the canopy (i.e., vertical and horizontal coordinates in the vine).

Statistical Analysis

The dependent variable for data analysis, *B. cinerea* cfu per flower, was transformed by natural log ($\log_n$) after the distribution of residuals of data, $\log_n$data and $\sqrt{}$data was assessed. The influence of the dispersal agent (honey bee, wind or control), plot, direction (North or South) and distance from inoculum zone, on the number of cfu per flower, was analysed by ANOVA. Dispersal gradients of $\log_n$ the number of cfu dispersed by honey bees per flower, with distance from the inoculum zone, were analysed by both linear regression and spline regression (Smith 1979) with two degrees of freedom (i.e., allowing for one direction of curvature) to better describe results.

For wind and control treatments, the mean number of cfu per flower at distance 0 m (in the inoculum zone) versus > 0 m (outside the inoculum zone) were compared by ANOVA, and log-linear regression analysis was carried out while excluding data at distance 0 m.

4.3 RESULTS

Meteorological details were obtained from HortResearch from their Riwaka station c. 500 m from the study site. The day was fine, maximum temperature 20°C, 7.8 sunshine hours and 106 km (Northerly) wind run.

After 8 h foraging, the mean number of *B. cinerea* cfu detected per artificially inoculated flower was 6.2×10^4 (N=48, standard deviation 4.2×10^4).

B. cinerea Incidence

B. cinerea cfu were detected from 100% of the 279 honey bee dispersal samples collected, compared with 55% of wind samples (N=75) and 12% of control samples (N=65). Flower numbers were approximately 500 per vine. Eighteen samples were positioned >2 m (2.1-2.3 m) from inoculum zones, because there had not been enough flowers at the required stage of opening within 2 m of the inoculum zone. Raw data are presented in Appendix II.

Summary Of Analysis

The ANOVA comparing dispersal agent, plot, row direction, and distance effects on $\log_n$ cfu/flower is presented in Table 4-2. There were highly significant effects of dispersal agent ($P < 0.001$), plot ($P < 0.001$), distance ($P < 0.001$) and distance versus agent ($P < 0.001$).

Table 4-2: Analysis of variance comparing the effects of dispersal agent (agent), plot, north or south row direction (direction) and linear regression with distance on the $\log_n$ number of *B. cinerea* cfu per flower (dispersal gradient). Degrees of freedom (df), sums of squares (SS), F values (F), F probability (Pr > F).

SOURCE OF VARIATION	DF	SS	MS	F	Pr > F
Dispersal Agent	2	2403.9	1201.9	2482.1	<0.001
Plot	5	13.1	2.62	5.41	<0.001
Agent vs. Plot	10	3.98	0.40	0.82	0.608
Direction (Nth vs Sth)	1	0.40	0.40	0.82	0.364
Direction x Plot	5	3.68	0.74	1.52	0.181
Dispersal Gradient	1	47.88	47.88	98.88	<0.001
Gradient vs. Agent	2	10.44	5.22	10.78	<0.001
Gradient vs. Plot	5	2.18	0.44	0.90	0.481
Gradient vs. Direction	1	0.38	0.38	0.78	0.378
Gradient shapes	6	63.99	10.67	22.51	<0.001
Means of slopes	2	49.10	24.55	51.82	<0.001

Honey Bee Dispersal

The relationship between honey bee dispersal of *B. cinerea* cfu to flowers with distance was highly significant ($P < 0.001$), using both log-linear and spline regression analyses. The spline regression was significantly different ($P < 0.05$), however, from the log-linear regression

(calculation in Appendix II). The raw data and spline regression curve for honey bee dispersal are presented in Figure 4-2.

Wind Dispersal

In the wind treatment, *B. cinerea* cfu were detected on 72% of samples in the inoculum zone (N=18), and on 49% of samples outside the inoculum zone (N=57). The number of cfu/flower transferred by wind in the inoculum zone (0 m) was significantly different ($P < 0.001$) from outside the inoculum zone (>0 m). There was a significant effect ($P < 0.01$) of the number of cfu per flower with distance, when data at distance 0 m (inoculum zone) were included in log-linear regression analysis, but no relationship ($P = 0.277$) when data at distance 0 m were excluded, i.e., the majority of the spread of data could be explained by the significant difference between samples in and outside the inoculum zone.

Control Treatment

In the control treatment, *B. cinerea* cfu were detected on 16% of samples in the inoculum zone (N=18) and 11% of samples outside the inoculum zone (N=47). The number of cfu per flower in the inoculum zone showed no significant variation ($P = 0.487$) from outside the inoculum zone. No significant effect ($P = 0.588$) was found between the number of cfu per flower with distance, regardless of whether data at distance 0 m were included or excluded in log-linear regression analysis, i.e., the spread of data (mostly 0 cfu's/flower) could be explained by random variation.

Table 4-3: Analysis of variance for each dispersal treatment. Comparing deposition gradients using log-linear regression analysis with and without including samples taken in the inoculum zone (distance 0 m). Degrees of freedom (df), sums of squares (SS), F values (F), F probability (Pr > F). The dependent variable, *B. cinerea* cfu/flower, was $\log_n$ transformed.

SOURCE OF VARIATION	DF	SS	MS	F	Pr>F
Honey Bee Dispersal					
Direction	1	0.40	0.40	0.83	0.362
Dispersal Gradient (data>0 m)	1	48.63	48.63	101.60	<0.001
Site	5	11.19	2.24	4.68	<0.001
Wind Dispersal					
0 m vs > 0 m	1	12.37	12.37	15.91	<0.001
Dispersal Gradient (data including 0 m)	1	8.48	8.48	10.06	0.002
Dispersal Gradient (data>0 m)	1	0.91	0.91	1.20	0.277
Site	5	5.57	1.14	1.47	0.213
Control (no dispersal)					
0 m vs > 0 m	1	0.065	0.07	0.49	0.486
Dispersal Gradient (data including 0 m)	1	0.082	0.083	0.62	0.434
Dispersal Gradient (data>0 m)	1	0.04	0.04	0.30	0.588
Site	5	0.33	0.07	0.47	0.796

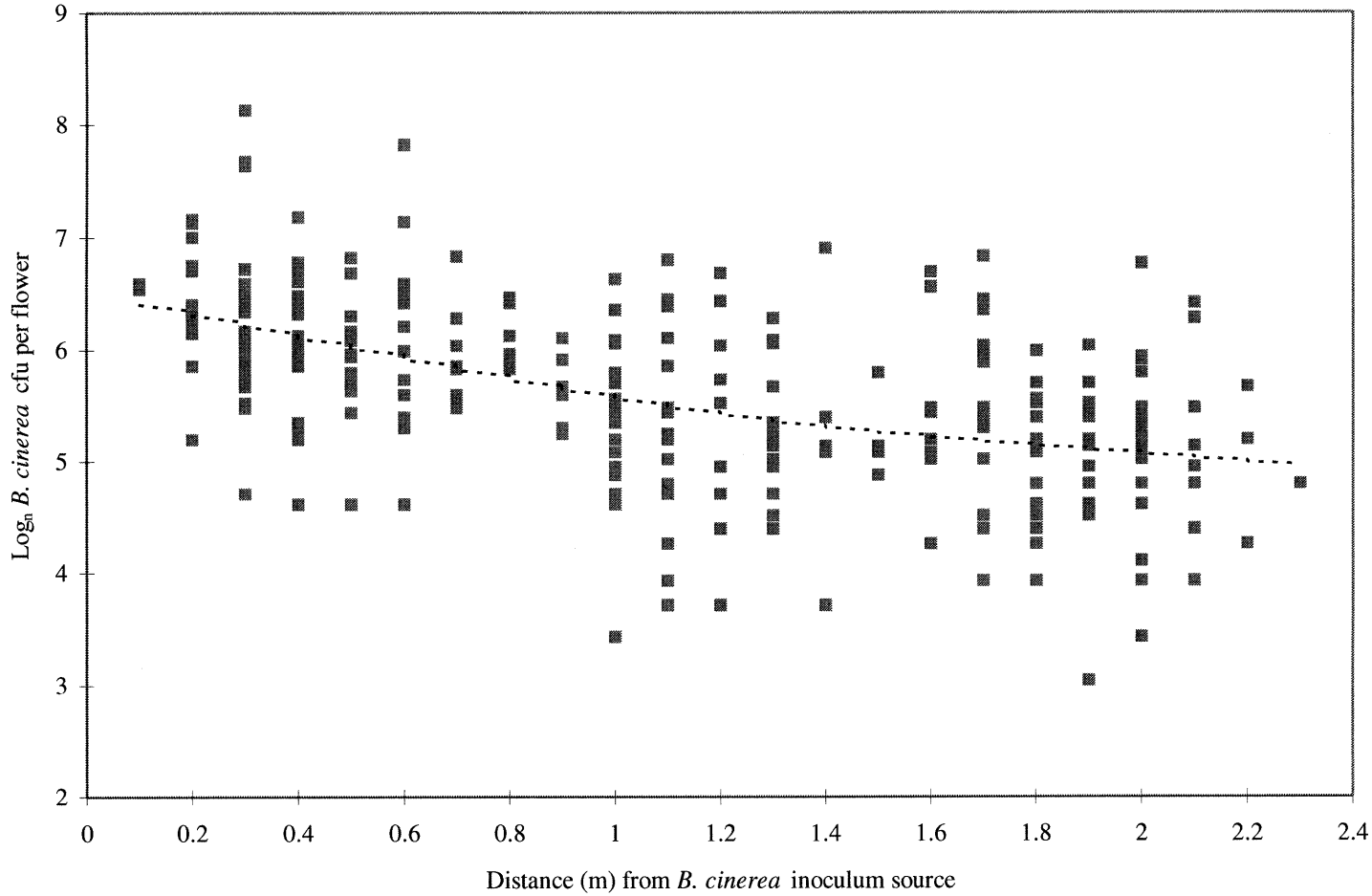


Figure 4-2: Honey bee dispersal of *B. cinerea* colony forming units per kiwifruit flower with distance from inoculum source. Dotted line is the fitted spline regression (significantly different from log-linear gradient $P < 0.05$).

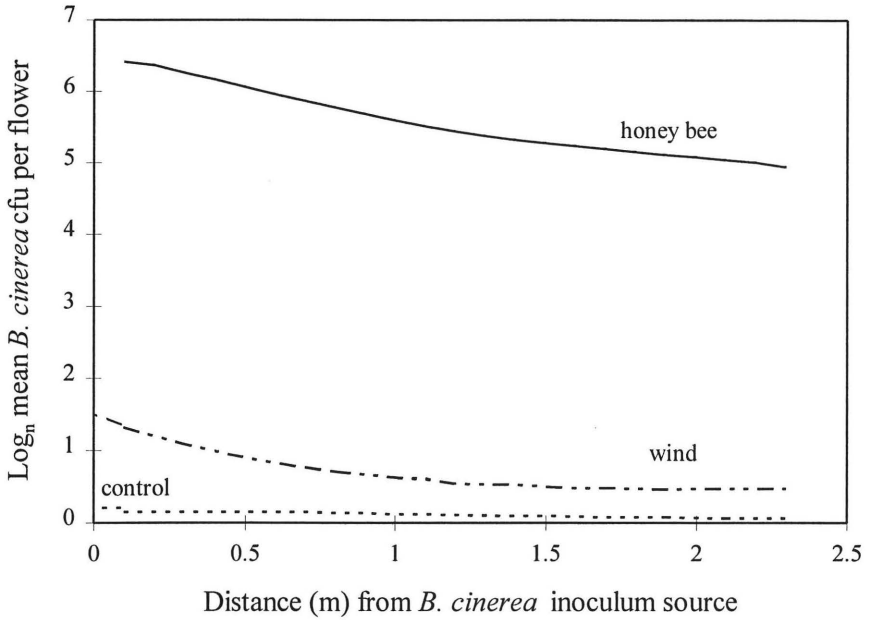


Figure 4-3: Fitted spline regression curves for honey bee and wind dispersal of *B. cinerea* colony forming units per kiwifruit flower with distance from inoculum source.

4.4 DISCUSSION

The first hypothesis, that the number of *B. cinerea* cfu per kiwifruit flower transferred by honey bees decreases with distance from a source of flowers contaminated with *B. cinerea* spores, was supported. The second hypothesis, that kiwifruit flowers within 2 m of a source of inoculated flowers become contaminated with a higher average number of *B. cinerea* cfu when visited by honey bees than similarly positioned flowers which are not visited by bees, was also accepted.

Insects Other Than Honey Bees

The observed frequency of foraging by insects other than honey bees was not considered sufficient to significantly add to spore transfer. Zero to five thrips had re-established themselves on flowers soon after plots had been sprayed with insecticide because the buds that opened after spraying were not protected. Similarly, Tomkins *et al.* (1992) reported a 45-74% reduction in thrips numbers on kiwifruit flowers 12 days after spraying with fluvalinate. Flowers in all treatments were observed carrying approximately equal numbers of thrips, and

therefore the effect of thrips on inoculum transfer was not considered important once significant differences between treatments were found.

Distance Effects On Dispersal

The results indicated that bees are more efficient vectors of *B. cinerea* cfu between kiwifruit flowers than wind under the wind conditions experienced on the day of this experiment. The incidence of *B. cinerea* on flowers varied widely between honey bee, wind and control treatments. *B. cinerea* cfu were detected on 100% of flowers exposed to foraging honey bees, compared to 55% of flowers in the wind treatment and 12% of flowers in the control treatment. The mean number of propagules dispersed with distance was also significantly higher for honey bee dispersal than wind dispersal and control treatments. Deposition by honey bees was in the order of two magnitudes greater than that by wind dispersal. The background level of *B. cinerea* detected on control samples was negligible. This demonstrated that inoculum transfer by wind had occurred; however, the degree to which mesh bags had impeded wind transfer of spores to flowers was unknown. There would probably be a disturbed airflow around such a shade cloth bag that would perhaps impede spore deposition. In future work, it would be interesting to estimate the reduction in the movement of airborne spores through shade cloth, so that deposition data could be accordingly adjusted, e.g., by measuring spore movement through mesh at varying wind speeds in a wind tunnel.

Site And Directional Effects On Dispersal

For the control and wind treatments, *B. cinerea* dispersal was measured only in one direction per site. With mean cfu per flower for each site pooled, North and South samples were not significantly different from each other. The slightly higher value for samples from the South may have been related to the northerly wind. The reliability of this analysis, however, was partially confounded due to the fact that site differences could not be separated from differences in direction of dispersal for the wind and control treatments. There was no significant difference between sites for the control treatment (0.796), an intermediate effect for the wind treatment ($P = 0.213$) and a significant effect for the honey bee treatment ($P < 0.001$). When treatment data were pooled within sites, however, site differences were highly significant ($P < 0.001$). This indicated that the effects of individual treatments were additive, thus increasing the certainty

that sites were significantly different. The interaction between dispersal agents versus plot was not significant (P = 0.608), i.e., the relationship between agents within each plot was common.

The significant effect of site with honey bee dispersal may have been due to the placement of bee hives and their distance from sites.

Honey Bee Dispersal

It was very interesting to find that the deposition gradient calculated for *B. cinerea* dispersal between kiwifruit flowers by honey bees, closely followed an exponential decline within two metres from the inoculum source. By fitting a spline to the data, however, the shape of the gradient differed significantly from an exponential decline ($P < 0.05$), due to the relative flattening out of the gradient with >1 m compared with <1 m distance from the inoculum source. The fact that the spline regression was significantly different from the log-linear regression at the 5% level, showed that most of the variation in the gradient was described by a log-linear line. This was not a completely satisfactory model, however, because of a systematic departure from the log-linear regression as the gradient dropped off.

Inoculum dispersal patterns resulting from transference by honey bee vectors could differ dramatically from airborne dispersal. When bees are such efficient vectors of *B. cinerea*, as has been demonstrated here, it would seem more probable that the dispersal pattern, resulting from their activity, would follow their foraging flight behaviour patterns. Honey bees are probably efficient vectors because they have the ability to transfer the pathogen from an infected flower directly to a new infection site, compared with the much lower probability of transference involved in airborne spore dispersal.

Dyer & Seeley (1985) described the distribution of distances flown by honey bees to natural flower sources from a hive in a northern temperate forest, as part of their foraging behaviour. The distribution pattern approximated a Ricker-type curve ($x_{t+1} = x_t \exp[r(1-x_t)]$ where $x>3$), with distribution distances increasing rapidly from the hive, reaching a peak, then decreasing, initially at a rapid rate but levelling off and becoming very gradual. The median foraging distance was 1.7 km from the hive, but distances of over 10 km were covered. This kind of foraging pattern would vary depending on the crop ecosystem and the alternate pollen and

nectar sources available. Presumably the peak foraging distance would shift with the changing quantity and quality of flower nectar and pollen sources. The pattern of dispersal of *B. cinerea* vectored by honey bees may follow this pattern rather than a simple negative exponential gradient (part of the Ricker-type curve does approximate a negative exponential curve). Therefore, the pattern of pathogen dispersal is likely to depend on honey bee foraging patterns, which will be influenced by the distance from the hive that the inoculum foci are positioned. The pattern and extent of *B. cinerea* dispersal will also depend on other variables such as the number of inoculum sources, number of vectors, probability of spore acquisition by vectors, spore load, probability of spore deposition on floral surfaces, duration of spore viability, etc.

It does not seem appropriate to draw conclusions about the shape of the dispersal curve in this experiment, since 2 m represents only a small distance in relation to the potential honey bee foraging flight distances. A small section of any curve will approximate a line, but conclusions cannot be drawn on the overall shape of dispersal patterns on the basis of this. It may be that the section of the dispersal pattern measured in this study may approximate a negative exponential, but the shape of dispersal outside of this range cannot be extrapolated.

The foraging behaviour of honey bees is inclined towards movement along rows compared with between rows (Goodwin & Steven 1993), which could have caused a 'funnelling' effect of inoculum dispersal compared with, for example, the fanning effect of wind dispersal. The non-uniform distribution of flowers and their variable attractiveness to bees (Goodwin 1986a), combined with variable spore loads on bees, may have accounted for the wide range in cfu counts at any one distance from inoculum zones.

Elmer *et al.* (1995) showed that fruit immediately adjacent to staminate vines at petal fall were externally contaminated with significantly more *B. cinerea* propagules compared to fruit adjacent to pistillate vines. They proposed that this was due to the large inoculum source found on staminate flowers and dispersal of that inoculum. Honey bees may be an important agent of inoculum dispersal between staminate and pistillate flowers.

Goodwin (1986b) demonstrated that the size of mature kiwifruit decreases with increasing distance from staminate vines, and attributed this to honey bee foraging behaviour. Deposition gradients could also be used to describe the amount of staminate pollen transfer with distance to pistillate flowers. This information could be used to calculate the greatest distance that pistillate vines should be situated from staminate vines within and between rows for adequate honey bee pollination.

Efficiency Of Wind Dispersal

The deposition gradient calculated for wind dispersal of *B. cinerea* to kiwifruit flowers decreased exponentially with distance from the inoculum source. The number of *B. cinerea* cfu deposited per flower within the inoculum zone was significantly higher than the number deposited outside the inoculum zone. When data from samples collected from the inoculum zone were omitted from regression analysis, inoculum dispersal to flowers was not significantly affected by distance from the inoculum zone, indicating that wind deposited detectable amounts of inoculum onlu within short distances (<0.1 m) of the source. Contamination of flowers in mesh bags in the inoculum zone, however, may have occurred during spore application to adjacent un-bagged flowers.

The low mean number of cfu/flower dispersed by wind may have been due primarily to reduced wind speed in the dense kiwifruit canopy, sheltered further by willow (*Salix matsudana*) hedges. Some characteristics of the architecture of kiwifruit vines trained on a T-bar system may also have minimised the efficiency of wind dispersal. Kiwifruit flowers are pendulous in form, spaced widely apart and trained on a high canopy, therefore the probability of spores settling or impacting on kiwifruit flowers while moving in air currents may be low. Also, spores would tend to be released downward below the canopy. In still air, *B. cinerea* spores sediment at a terminal velocity of approximately 10 mm per second, when spore weight is balanced by friction (Gregory 1976). Only turbulence in air currents below the canopy would drive some spores back up amongst the flowers. Wind dispersal of *B. cinerea* spores to host crops, such as grapes and strawberries, may be considerably higher where flowers and fruit are less sheltered from wind and more densely spaced.

Exponential models have fitted deposition gradients of many dry, airborne (Aylor 1990; McCartney 1994) and splash-dispersed (Fitt & McCartney 1986) spores. Deposition gradients of wind-dispersed particles can extend for distances from a few centimetres to hundreds of metres depending on wind, particle size, position and size of the source (Fitt *et al.* 1989). Deposition gradients for plant pathogens measured within the foliage of crops usually extend for distances in the order of a few metres (McCartney 1994).

For the control treatment (no dispersal agent), the relationship between cfu per flower with distance was not significant. The spread in data from samples collected from the inoculum zone was not significantly different from that outside the inoculum zone. This demonstrated that enclosing flowers in waxed paper bags prevented inoculum transfer. The low levels of *B. cinerea* cfu in control treatment samples may suggest a widespread presence of low levels of inoculum in the orchard.

Infection Gradients And Inoculum Sources

Deposition gradients do not necessarily reflect infection gradients. Measurement of infection gradients requires further integration of the life cycle of the fungus and of the environment. Fully developed insect and aerobiological dispersal models that take into account host infection can supply a logical basis for the strategies needed to control epidemics.

Strength Of Inoculum Source

After 8 h foraging, the number of cfu remaining per inoculated flower varied widely, both within, and between plots (10 000 - 205 000 cfu per flower). If inoculated flowers had been sampled immediately after spore application, then the strength of the inoculum source at each plot may have been useful in describing dispersal analysis. In practice, the differences amongst the number of propagules available for dispersal, and amongst the number actually dispersed was very large. The propagules remaining after 8 h foraging may have been on parts of the flowers that were not contacted by foraging bees, or on flowers that were not attractive to bees, e.g., when there was no available pollen. The number of spores applied per flower was not calibrated, so large differences between flowers were expected, but the overall source strength

per plot was designed to be very high, so that it would not be a limiting factor in spore pick up and dispersal.

Spore Transfer Between Foraging Trips And Between Bees

In the hive, honey bees fail to thoroughly clean their bodies between foraging trips, and viable pollen can be transferred between foragers as they contact each other in the hive (Free *et al* 1991). Free & Williams (1972) found that between 2 000 and 5 000 pollen grains commonly occurred on the bodies of newly-emerged honey bees before they ever foraged. Given that honey bees contaminated with fungal spores would also be unlikely to clean all spores from their bodies in the hive, spore transfer between bees seems very likely. Individual bees typically restrict their foraging to a small area (Goodwin 1986b), so spore transfer between bees could increase the area over which a disease is spread. External spore load, ease of spore transfer between bees and floral surfaces, and the length of time spores remain viable would be major determinants in whether or not spore transfer between foragers is important in the vectoring process. Within-hive spore transfer could be investigated by infecting a proportion of foraging bees with *B. cinerea* spores, marking these bees, and sampling unmarked bees in the hive at varying time intervals. Newly emerged bees that have not begun foraging, and therefore could not have visited a *B. cinerea* inoculum source, can be identified and marked easily in the hive (Donovan pers. comm.) to be used as indicators of spore transfer.

Future Work

The proportion of inoculum that bees transfer with consecutive flower visits after inoculum acquisition could be investigated in future studies. This could then be related to the 'inoculum strength' of natural sources in the field which bees visit, the frequency of such visits, and the spatial foraging patterns of bees to more fully describe the vectoring process.

B. cinerea cfu were detectable on unbagged flowers out to the maximum distance (2.3 m) from the inoculum zone that was sampled. Future work could measure dispersal at greater distances, both within and between rows of kiwifruit. The deposition gradient calculated for inoculum dispersal by bees in this experiment could be used to estimate the distance from the inoculum zone where inoculum levels would be likely to be at the background level.

Shykoff & Bucheli (1995) investigated patterns of pollinator visitation through populations of the white camion, *Silene alba*, containing plants which were either healthy or diseased with the anther smut fungus, *Ustilago violacea* (= *Microbotryum violaceum*), by tracing the spread of fluorescent dye powder. This technique could be useful to study the pattern of honey bee movement in kiwifruit orchards and then related to *B. cinerea* transfer using deposition gradient data. Releasing a marked strain of *B. cinerea* (e.g., dicarboximide-resistant) could also be a useful way of tracking inoculum transfer, thus eliminating the masking effect of background inoculum.

It could be useful to develop this study further by investigating the effect of applying a biological control agent or fungicide to flowers via bees inoculated at the hive, as in *Erwinia amylovora* control (Johnson *et al.* 1993; Peng *et al.* 1992; Thomson *et al.* 1992) to reduce inoculum build-up at flowering in kiwifruit orchards.

4.5 CHAPTER SUMMARY

- Honey bees vectored *B. cinerea* to kiwifruit flowers. Flowers visited by honey bees carryied higher numbers of cfu than flowers positioned similarly in relation to the inoculum source but, protected from bee visitation.
- Wind dispersal of *B. cinerea* cfu between kiwifruit flowers was negligible compared with dispersal by honey bees.
- The number of *B. cinerea* cfu transferred by honey bees to kiwifruit flowers decreased with distance from a source of inoculum.
- Honey bee dispersal of *B. cinerea* cfu between kiwifruit flowers closely followed a negative exponential rate within 2 m of an inoculum source.

5. RELATIONSHIP BETWEEN SURFACE CONTAMINATION OF FRUIT BY *B. CINEREA* AT PETAL FALL WITH MID FRUIT AND MATURE FRUIT STAGES

5.1 INTRODUCTION

The transfer of *B. cinerea* inoculum to kiwifruit flowers by wind and insects, such as honey bees, may influence the amount of external contamination of fruit during the season. Viable *B. cinerea* propagules have been isolated from the external surfaces of kiwifruit leaves, canes, fruit and flowers (Elmer *et al.* 1993; 1992). *B. cinerea* conidia may remain ungerminated on plant surfaces for long periods, impeded by lack of moisture, nutrients or by microbial or plant antagonism (Blakeman 1980). Propagules may survive through to harvest, or infect and sporulate on attached senescent flower parts to cause secondary contamination of the surface of fruits. Elmer *et al.* (1995; 1994) showed that spores on the fruit surface may be an important source of inoculum for redistribution in the picking bag and picking wound contamination.

The aim of this study was to determine if the number of *B. cinerea* spores applied to the fruit surface at petal fall influenced the number of propagules on the fruit surface later in its development. A relationship between propagule number at early and late development of fruit would indicate the potential for honey bees spreading inoculum during flowering to influence *B. cinerea* epidemiology later in the season.

5.2 METHODS

Study Site

This experiment was undertaken in December 1992 in a mature commercial kiwifruit orchard (Stevens) cv. Hayward in Riwaka trained on a T-bar system with rows 5 m apart and irrigated with under vine sprinklers. Six rows of kiwifruit were available for this study. Vines in these rows received no fungicide sprays during the experiment, otherwise standard cultural and pest control practices were applied.

B. cinerea Spore Application Treatment

A virulent isolate of *B. cinerea* was obtained in 1992 from an infected kiwifruit cv. 'Hayward' in coolstorage at the HortResearch Research Station at Riwaka. Slices of healthy kiwifruit were inoculated by transferring infected kiwifruit tissue to the healthy slices with a sterile metal loop. The inoculated slices of kiwifruit were sealed in plastic bags on trays and sporulating cultures were produced after ten days incubation in natural light. Growth temperatures were allowed to fluctuate with ambient temperature, which varied from 18-25°C.

Spores of *B. cinerea* were harvested from kiwifruit slices in sterile distilled water containing 0.01% (v/v) Tween 80. Pieces of mycelium, fruit hairs and seeds were removed by filtration through two layers of Whatman No. 105 lens tissue. The concentration of the spore suspension was measured using a haemocytometer, and the initial suspension was then diluted to give 1 litre of three spore suspensions ($1x10^6$, $1x10^5$ and $1x10^4$ spores ml^{-1}). The volume of spore suspension that was applied per fruitlet, using a hand-held puff-sprayer (designed for kiwifruit artificial pollination research), was calculated by separately weighing 20 fruitlets before and after a spray with one puff action of the sprayer. This made fruitlets wet, but with no liquid run-off. The sprayer applied, on average, 0.20 ml (+/- 0.06 ml) suspension per fruitlet. From the lowest to highest concentration of spore treatment, this correlated to fruitlets being sprayed with approximately 2×10^3, 2×10^4 and 2×10^5 spores per fruitlet, respectively. The control solution consisted of sterile distilled water plus 0.01% (v/v) aqueous Tween 80.

Experimental Design And Fruitlet Inoculation

Five replicate plots were selected in the study site, each consisting of one pistillate vine in full flower, in the area between two support posts (3 m x 2 m). Each plot was separated by at least three vines within a row and one vine between rows to minimise interplot interference. On 10 December 1992, the three spore application treatments and control treatment were each applied at random to three samples of five fruit per plot using a puff-sprayer, beginning with the control followed by the increasing concentrations of spore suspensions. The sprayer was rinsed thoroughly with sterile distilled water between changes in spore suspensions, and flowers were sprayed with one of the four concentrations of spore suspension. Colour-coded tags were tied at the stem base of each fruit to identify treatments.

Sample Collection And *B. cinerea* Detection

One sample (of five fruit) per treatment, per plot, was harvested at each of the three sampling times: 'Petal fall' (10 December 1992), 'mid fruit' (15 January 1993), and 'mature fruit' (8 April 1993). Fruit were cut from the vine with secateurs at the pedicel and transferred into single layer plastic kiwifruit 'Plix' trays with tweezers. Samples were transferred to 0°C cool storage pending further processing.

B. cinerea propagules were dislodged from the surface of fruitlets using a method slightly modified from Fermaud *et al.* (1994). To dislodge *B. cinerea* propagules from the hairy fruit cuticle, each sample of five fruitlets was washed in bulk by shaking the fruit vigorously for 120 s in 100 ml sterile distilled water plus 0.01% (v/v) Tween 80 and then in 100 ml of sterile distilled water only. Fruitlets were removed and each suspension was filtered separately, first through Whatman 105 lens tissue to remove fruit hairs and other large particles of debris, and then vacuum filtered through a Millipore filter (5 μm pore size) to retain *B. cinerea* conidia (6-10 μm diameter). For petal fall and mid fruit samples, 2-4 millipore filters were required to filter each sample, while up to five filters were required with samples at time 4 months. Propagules were re-suspended from filters in 10 ml sterile distilled water containing 0.01% (v/v) Tween 80 in a Universal bottle by agitating samples vigorously for 60 s by hand. The suspensions were diluted serially (1:1, 1:10, 1:100, 1:1000, 1:10 000), and one 75 μl aliquot per dilution was pipetted onto *Botrytis*-selective medium (Appendix I) in Petri dishes. All equipment was rinsed with sterile distilled water between samples.

Plates were incubated and the number of mycelial colonies assessed as described in Section 2.2.1. Results were expressed as mean number of *B. cinerea* cfu per fruit.

Statistical Analysis

The dependent variable, *B. cinerea* colony forming units (cfu) per fruit, was transformed by natural log ($\log_n$) after the distribution of residuals of cfu, $\log_n$cfu and $\sqrt{}$cfu was assessed. Log_n cfu per fruit were compared by plot, spore application treatment, and time, using analysis of variance. An orthogonal contrast was specified for the spore application factor (control versus spore treatments) with linear and quadratic curvature components specified for the spore

treatments (2×10^3, 2×10^4 and 2×10^5) and time factor, which was analysed relative to days after initial spore treatment application.

5.3 RESULTS

B. cinerea sporulation was observed on attached flower parts on inoculated and some uninoculated fruitlets five days after spore application. The mean numbers of *B. cinerea* cfu per fruit for each spore application treatment at each stage of fruit growth are summarised in Table 5-1. The analysis of variance of treatment effects is presented in Table 5-3. A summary of the significance of interaction effects are presented in Table 5-2. Raw data are presented in Appendix II.

Table 5-1: Log_n the mean number of *B. cinerea* colony forming units per fruit, per spore inoculation treatment, at initial (petal fall), mid fruit and mature fruit sample times (back-transformed values in parentheses).

	GROWTH STAGE			
Spores Applied Per Fruitlet	**Petal fall (time 0) 10 December 1992**	**Mid fruit 15 January 1993**	**Mature fruit 8 April 1993**	**Mean/ Spore Application Treatment**
Control (0)	5.92 (3.7×10^2)	8.33 (4.2×10^3)	8.11 (3.3×10^3)	7.45 (1.7×10^3)
2×10^3	7.56 (1.9×10^3)	9.04 (8.4×10^3)	8.95 (7.7×10^3)	8.52 (5.0×10^3)
2×10^4	8.08 (3.2×10^3)	9.66 (1.6×10^4)	8.57 (5.3×10^3)	8.77 (6.4×10^3)
2×10^5	10.37 (3.5×10^4)	10.09 (2.4×10^4)	9.49 (1.3×10^4)	9.98 (2.2×10^4)
Mean/ Time	7.98 (2.9×10^3)	9.28 (1.1×10^4)	8.78 (6.5×10^3)	

LSD (5%) for comparing main effect means: spore treatments = 0.79, sample time = 0.65

Table 5-2: Significance of interactions.

Source of Variation	
Spore Application (control vs. treated) vs. Time (linear component)	*
Spore Application (linear component in treated) vs. Time (linear component)	*
Spore Application (control vs. treated) vs. Time (quadratic component)	n.s.
Spore Application (quadratic component) vs. Time (linear component)	n.s.
Spore Application (linear component in treated) vs. Time (quadratic component)	n.s.

* = significant at the 5% level

Table 5-3: Analysis of variance comparing effects of spore treatments and fruit growth stage on the mean number of *B. cinerea* cfu per fruit. Sums of squares (SS), degrees of freedom (df), F values (F), F probability (Pr > F).

SOURCE OF VARIATION	DF	SS	MS	F	Pr > F
Replicate Plots	4	3.724	0.931	0.89	0.480
Spore Application					
Control vs. Treated	1	30.144	30.144	28.68	<.001
Linear component in Treated	1	16.116	16.116	15.33	<.001
Quadratic component in Treated	1	2.308	2.308	2.20	0.146
Time					
Linear component	1	2.963	2.963	2.82	0.100
Quadratic component	1	14.229	14.229	13.54	<.001
Spore Application vs. Time					
Spore Application (control vs. treated) vs. Time (linear component)					
	1	5.237	5.237	4.98	0.031
Spore Application (linear component in treated) vs. Time (linear component)					
	1	5.244	5.244	4.99	0.031
Spore Application (control vs. treated) vs. Time (quadratic component)					
	1	2.035	2.035	1.94	0.171
Spore Application (quadratic component in treated) vs. Time (linear component)					
	1	0.000	0.000	0.00	0.992
Spore Application (linear component in treated) vs. Time (quadratic component)					
	1	1.827	1.827	1.74	0.194
Deviations	1	2.710	1.752	1.67	0.203
Residual	44	46.251	1.051		
Total	59	131.828			

There was no effect of replicate plots (P = 0.480), which showed that the spread of data was similar within each plot.

There was a significant difference (P < 0.001) between the number of propagules per fruit on control versus spore-treated fruit. The linear contrast between spore treatments was significant

($P < 0.001$), demonstrating that the relationship varied over time, with the differences being less pronounced at the mature fruit stage. The quadratic contrast between treatments was not significant, i.e., the curvature in the line was not significantly different from zero.

The contrast between the number of propagules per fruit over time had a significant linear component ($P = 0.100$) at the 10% level, showing that the relationship varied slightly over time. The quadratic curvature component with time was highly significant ($P < 0.001$), i.e., the curvature in the line was significantly different from zero. This described the greater number of propagules per fruit at mid fruit stage ($\log_n$ 9.28) than at petal fall ($\log_n$ 7.98) and mature fruit ($\log_n$ 8.78).

Interactions

The interaction between spore treatment (control versus treated) and time (linear component) was significant ($P = 0.031$), i.e., the difference between control versus treated changed over time. The interaction between spore treatment (linear in treated) and time (quadratic component) was significant ($P = 0.031$), i.e., the relationship between cfu per fruit and spore application rate varied with the quadratic component of time. The steepest spore application slope was at petal fall compared to the shallowest slope at the mature fruit stage. There were no significant relationships between (i) spore treatment (control vs. treated) and time (quadratic), (ii) spore treatment (curvature in treated) and time (linear), and (iii) spore treatment (linear in treated) and time (quadratic).

5.4 DISCUSSION

Viable propagules were detected on the fruit surface in the control treatment at the three growth stages sampled, indicating that inoculum occurred naturally. There were relatively low natural levels of inoculum on the fruit at petal fall. By mid January, however, the natural levels of propagules had increased markedly, while the cfu per fruit on inoculated fruit did not increase at the same rate. At the highest inoculation rate, there was no significant difference between numbers of cfu per fruit from petal fall to mid fruit. This may indicate that loss of spore viability of spores applied at petal fall was off-set by spore production or spore trapping.

Spore application at petal fall stage significantly increased the spore load on these fruits at the three growth stages sampled. This suggests that honey bee dispersal of *B. cinerea* inoculum to flowers could influence the number of viable *B. cinerea* propagules contaminating fruit surfaces during the season. Such contamination could occur either through surface contamination at fruitlet stage, or through the establishment of secondary infections on senescing and necrotic flower tissues. Secondary infections would give rise to higher levels of inoculum immediately adjacent to the fruit, which would have a high probability of being trapped on the fruit surfaces.

In each one-vine plot, 45 fruitlets were inoculated with spores, from which 15 fruitlets were immediately sampled. A total canopy proliferation of *B. cinerea* due to spore application to the 30 remaining fruitlets was not expected. In this experiment there may have been a natural overall increase in the amount of inoculum in plots to mid fruit (as indicated by the increase in spore load on control fruits), as well as localised profuse sporulation on senescing and necrotic flower parts that were sprayed with *B. cinerea* spores.

Elmer *et al.* (1992) showed that the number of airborne spores fluctuated widely throughout one season, with distinctive peaks being detected prior to bud burst, during flowering and in February. Elmer *et al.* (1994) suggested that high spore production in the vine may result in many spores on the fruit surface before harvest, thus increasing the likelihood of spores being transferred to the picking scar. It has been proposed that the hairy surface of the Haywood variety of kiwifruit may trap and accumulate *B. cinerea* spores during the season, and may provide some protection for the spores from environmental extremes (Elmer *et al.* (1995); Fermaud *et al.* 1994). A general increase in spore production in vines to the mid fruit stage and the trapping of spores between fruit hairs, could account for the increase in cfu per fruit on control fruit in this experiment. Immediately, spores produced on fruitlets sprayed with inoculum, may have been trapped by the fruit hairs on the same or neighbouring fruits during aerial dispersal. Elmer *et al.* (1995) found a slight increase in the number of *B. cinerea* cfu which naturally contaminated kiwifruit from petal fall (735/cm^2 of fruit surface) to midfruit (February) (772/cm^2), then a decrease to harvest (April) (228/cm^2). The surface area of fruit

was not taken into account in this experiment, but would have caused a greater decline in cfu/fruit at harvest, and a slight decline at mid fruit.

5.5 CHAPTER SUMMARY

- There was a significant effect of applying *B. cinerea* spores to fruitlets in increasing the number of *B. cinerea* cfu carried externally on fruit during the season.
- A greater number of *B. cinerea* cfu per fruit were found at the mid fruit stage than at the fruitlet or mature fruit stage.
- There was a general increase in the number of *B. cinerea* cfu per fruit from fruitlet to harvest stage.
- High natural levels of *B. cinerea* inoculum were trapped on fruit surfaces.

GENERAL DISCUSSION

The role of honey bees as vectors of *B. cinerea* to kiwifruit flowers and the influence of honey bee vectoring on *B. cinerea* epidemiology were investigated. In this chapter, results and implications of this research are discussed in relation to *B. cinerea* epidemiology. *B. cinerea* epidemiology is summarized in Figure 6-1.

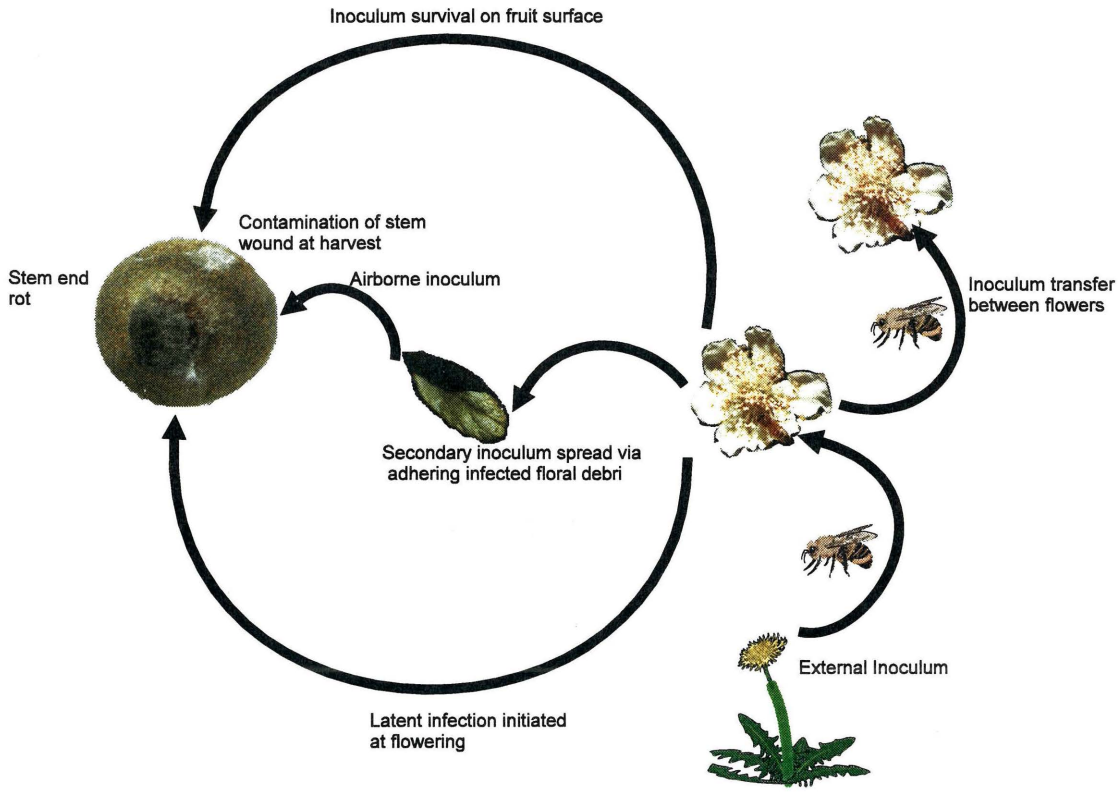


Figure 6-1: Epidemiology of *B. cinerea* stem-end rot of kiwifruit.

External Inoculum

Honey bees from colonies placed in kiwifruit orchards that forage on nectar sources outside orchards, could acquire *B. cinerea* spores from infected plant material. In Chapter Two, it was demonstrated that honey bees foraging on manuka flowers situated within foraging range of several kiwifruit orchards were contaminated with *B. cinerea* propagules. It was not demonstrated that bees acquired the disease inoculum from the manuka flowers. Plant species which host *B. cinerea* and flower at the same time as kiwifruit, however, such as strawberry (*F.*

ananassa), marrow (*Cucurbita pepo*), citrus (*Citrus* spp.) and rose (*Rosa* spp.), could act as inoculum sources for foraging bees. If the honey bees subsequently changed to collecting kiwifruit pollen, or if they transferred inoculum to kiwifruit-foraging bees in the hive, then inoculum could be transferred to kiwifruit flowers. If *B. cinerea* propagules can remain viable in bee hives overnight, then the location of colonies before their use for kiwifruit pollination may be a factor in the spread of inoculum to kiwifruit flowers. Boylan-Pett *et al.* (1991) showed that the transfer of pollen-borne blueberry leaf mottle virus occurred between honey bees in colonies, and that the disease remained infectious for at least 10 days within colonies. The temperature in bee colonies is a constant 34°C in summer weather (Root 1983) and although *B. cinerea* spore germination decreases quickly above 26°C, spore viability is not seriously affected at 40°C (Coley-Smith 1980). If *B. cinerea* can survive in bee hives that are transported between orchards, then the spread of dicarboximide-resistant strains of the fungus (Beever *et al.* 1989) could also be facilitated.

Inoculum Transfer Between Flowers

Once initial foci of infection are established on kiwifruit flowers during early flowering, perhaps by transfer of inoculum by bees from another flowering plant species, or by airborne inoculum, then spread of inoculum between flowers may occur. Results from Chapters Two, Three and Four supported the hypothesis that honey bees carry *B. cinerea* inoculum naturally in the field and are capable of transferring inoculum between kiwifruit flowers. This work resulted in the development of methods for collecting foraging honey bees directly from flowers and isolating viable *B. cinerea* propagules from the external surfaces of bees. The research described in Chapter Two showed that 87% of honey bees foraging on kiwifruit flowers carried viable *B. cinerea* propagules, while the mean numbers of *B. cinerea* propagules carried ranged between zero and 320 cfu per bee (s.d. 30). The research described in Chapter Three showed that honey bees picked up large numbers of *B. cinerea* cfu (mean 4×10^3, range 2.6×10^2 - 1.6×10^4 cfu per bee) while foraging on flowers that had been artificially contaminated with spores. Measurement of the dispersal of *B. cinerea* propagules between kiwifruit flowers (Chapter Four) showed that honey bees were effective inoculum vectors over short distances. All flowers available for honey bee visitation were contaminated with *B. cinerea* within 2.3 m of an inoculum source. The number of cfu dispersed to flowers by honey bees was significantly

greater than that dispersed by wind. Honey bees may, therefore, facilitate rapid and broad spread of *B. cinerea* to kiwifruit flowers.

Inoculum Persistence To Harvest

Infection of flowers with *B. cinerea* may influence stem end rot in several ways. First, latent infections initiated at flowering can contribute directly to stem-end rot in storage (Bisiach *et al.* 1984; Elmer *et al.* 1992). Second, leaf and cane lesions can develop from secondary spread via adhering debris from infected flowers (Pennycook 1985), thus increasing the total inoculum levels in orchards (Elmer *et al.* 1992; 1994) and risk of stem wound infection. Third, flower infections may increase the contamination of fruit surfaces with adhering flower tissues (Elmer *et al.* 1992), thus increasing the risk of stem wound infection during harvesting procedures. This hypothesis was supported by the study described in Chapter Five, which showed that applying *B. cinerea* spores to fruit at petal fall stage significantly increased the number of *B. cinerea* cfu on fruit surfaces later in the season. When spores were applied artificially at petal fall, there was a significant increase in the mean number of *B. cinerea* cfu per fruit both one month and four months (mature stage) after application, and the mean number of cfu isolated from inoculated fruit were significantly higher than from non-inoculated fruit.

Future Work

The research conducted in this thesis laid the ground work for future experimentation. Great scope remains to explore the dispersal of *B. cinerea* by honey bees to both kiwifruit flowers and other host crops. Research is justifiable on the basis of the pathogen's international economic importance as well as the fact that honey bees are common pollinators of many crops in which *B. cinerea* grey mould or storage rot of the fruit is a major component of economic loss. Future experiments could focus on the hypothesis that *B. cinerea* epidemiology in kiwifruit orchards is influenced by honey bees. This hypothesis gained support from the findings in this study that (i) honey bees carry *B. cinerea* inoculum naturally while foraging on kiwifruit flowers, (ii) honey bees are capable of picking up viable propagules while foraging on contaminated kiwifruit flowers, (iii) honey bees transfer more viable propagules between kiwifruit flowers than wind transfer, and (iv) surface contamination of fruit during the season may be influenced by initial inoculum levels at flowering. Important factors to be investigated in future studies

include: The length of time that *B. cinerea* propagules remain viable while carried by bees; The number of propagules required to initiate flower infection; Honey bee visitation of natural *B. cinerea* inoculum sources in kiwifruit orchards; The survival of inoculum in bee hives.

Elmer *et al.* (1993) suggested that strategies which reduce *B. cinerea* spore numbers in kiwifruit vines at flowering, and minimise the number of spores on fruit surfaces and senescent debri at harvest, could reduce stem-end rot in storage. It is not realistic to remove the vector from the kiwifruit system, because honey bees are necessary for adequate and cost-effective kiwifruit pollination. Therefore, the factors that promote the spread of *B. cinerea* by honey bees to kiwifruit flowers must be better understood so possible means of decreasing the spread of the fungus can be devised.

To control *B. cinerea* infection and inoculum build-up during kiwifruit flowering, a microbial biological control agent, which lives either as a saprophyte on the host plant surface or as a non-pathogenic parasite, could be applied to flowers. Honey bees could provide an economical and nonintrusive means of conveying biological control agents to kiwifruit flowers, perhaps by inoculuating foraging bees as they exit the hive. To be effective, the use of microbial fungicides, like conventional fungicides, must be critically timed, uniformly distributed, and available in appropriate dosage to the targeted host species. Honey bees would provide a readily available vector in kiwifruit orchards, where colonies can be easily manipulated and transported and where their foraging areas are, to a large extent, manageable. Honey bees have been used successfully on a field scale to transfer the biological control agents *Pseudomonas fluorescens* and *Erwinia herbicola* to apple and pear flowers to control fire blight (*E. amylovora*) (Johnson *et al.* 1993; Thomson *et al.* 1992) and *Gliocladium roseum* to strawberry flowers to control *B. cinerea* (Peng *et al.* 1992). Honey bees have also been used successfully to disseminate *Heliothis* nuclear polyhedrosis virus (HNPV) to crimson clover (*Trifolium incarnatum*) flowers in the field for the control of *Helicoverpa* spp. and *Heliothis* spp. insect pests (Gross *et al.* 1994). An advantage of using biological control agents of *B. cinerea* in kiwifruit over fungicide application, is that there would be no added build-up of resistance to dicarboximide sprays. The extent to which honey bee dispersal of biological control agents would be applicable for use in *B. cinerea* management would depend on an improved

understanding of the role of honey bees in the epidemiology of *B. cinerea* in kiwifruit, and the availability of effective biological control agents to attack the fungus.

It has been suggested in the literature that *B. cinerea* is spread mainly by wind and splash dispersal. Although this hypothesis may be reasonable in certain cases, there is need for the recognition that honey bees (as well as other insects) may be as, or more important, than wind at dispersing *B. cinerea* inoculum to host plants. It may be that the importance of insects as vectors of fungal plant pathogens in nature has been underestimated because it has been overshadowed by the generality of wind and water dissemination of fungal spores. More fungal diseases may be shown to be associated with insect vectors in the future. Such knowledge would not only improve our understanding of these diseases, but could help us control some of the diseases by focusing our attention not only on the pathogen but also on vectors of the pathogen. Progress in this area will require more cooperation between plant pathologists and entomologists and more interdisciplinary education for each speciality.

ACKNOWLEDGEMENTS

Thank you Roy and Bruce for your supervision and to my associate supervisor Phil Elmer for proposing the initial research idea and a window of opportunity. Thanks to my technical advisor Nick Pyke for invaluable practical advice. Thank you also to Warren Pulford (sorry about the bee stings!), Judith Pay and Kirsty Boyd-Wilson for technical advice.

I gratefully acknowledge the financial support that was provided by scholarships from HortResearch, Dow Elanco, and Leornard Condell during my studies.

To Craig Phillips, who managed to wrestle the first draft of my thesis from me and set the ball rolling - and to Hamish Macnab, for tirelessly editing my final drafts - your enthusiasm and confidence in my work were all important.

I would like to acknowledge my workmates at AgResearch whom helped me through many of my final hurdles. In particular, a huge thank you to Geoff Hurrell, Dave Saville and David Baird for helping me with data analysis, Ian Harvey for fungal taxonomic advice and Ian Laurenson for absolutely superb computer support.

To my wonderful family and friends, especially to my mother Fran, my boyfriend Craig, sisters Erin and Jenny, brother David, Zoe, Diana, Herm, Hamish, Lucette, Yoz, Robin and Oak- your happy smiles, encouragment and understanding meant everything.

REFERENCES

Agrios, G. N. 1980. Insect involvement in the transmission of fungal pathogens. In: *Vectors of Plant Pathogens*. Edited by Harris, K.F. and Maramorosch, K. Academic Press, New York, USA. pp. 293-317.

Ainsworth, G. C., James, P. W. and Hawksworth, D. L. 1971. *Dictionary of the Fungi.* Sixth edition. Commonwealth Agricultural Bureaux, Surrey, England. p. 663.

Alexander, G. P. 1986. Kiwifruit - Male Vines: Selection, Distribution and Husbandry Techniques. *Kiwifruit Pollination Seminar Proceedings*, Ministry of Agriculture and Fisheries, Tauranga, New Zealand, October 1986. p. 1-20.

Anon. 1995. *Situation and Outlook for New Zealand Agriculture*. Edited by Whittington, B. G. P. Publications, Wellington, New Zealand. 133 p.

Anon. 1973. A guide to the use of terms in plant pathology. *Phytopathological Papers* No. 17. 55 p.

Aylor, D. E. 1990. The role of intermittent wind in the dispersal of fungal pathogens. *Annual Review of Phytopathology* **28**: 73-92.

Aylor, D. E. 1978. Dispersal in time and space: aerial pathogens. In: *Plant Disease, An Advanced Treatise* (Volume II). Edited by Horsfall, J. G. and Cowling, E. B. Academic Press, New York. pp. 159-180.

Beever, D. 1979. Botrytis storage rot of kiwifruit. *Kiwifruit Seminar Proceedings*, Tauranga, 1979. Ministry of Agriculture and Fisheries Publication. pp. 29-36.

Beever, R. E., Laracy, E. P. and Pak, H. A. 1989. Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. *Plant Pathology* **38**: 427-437.

Beever, R. E. and Brien, H. M. R. 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. *New Zealand Journal of Agricultural Research* **26**: 391-400.

Beever, R. E. and Parkes, S. L. 1993. Mating behaviour and genetics of fungicide resistance of *Botrytis cinerea* in New Zealand. *New Zealand Journal of Crop and Horticultural Science* **21**: 303-310.

Bisiach, M., Minervini, G. and Vercesi, A. 1984. Biological and epidemiological aspects of the kiwifruit rot, caused by *Botrytis cinerea* Pers. *Rivista Patologia Vegetale* **20**: 38-55.

Blakeman, J. P. 1980. Behaviour of conidia on aerial plant surfaces. In: *The Biology of Botrytis*. Edited by Coley-Smith, J. R., Verhoeff, K. and Jarvis, W. R. Academic Press, New York. pp.115-151.

Blanchet, P. 1990. Kiwifruit (*Actinidia deliciosa* Chev.) pollination: honeybee behaviour and its influence on the fruit. *Acta Horticulturae* **282**: 105-111.

Bos, L. 1983. *Introduction to plant virology.* Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands 160 p.

Boylan-Pett, W., Ramsdell, D. C., Hoopingarner, R. A., and Hancock, J. F. 1991. Honeybee foraging behaviour, in-hive survival of infectious pollen-borne blueberry leaf mottle virus and transmission in highbush blueberry. *Phytopathology* **81**: 1407-1412.

Brook, P. J. 1990a. Review: *Botrytis cinerea* and fruit rot of kiwifruit, *Actinidia deliciosa.* Report to the New Zealand Kiwifruit Marketing Board, 99 p.

Brook, P. J. 1990b. Diseases of kiwifruit. In: *Kiwifruit: Science and Management.* Edited by Warrington, I.J. and Weston, G.C. Ray Richards Publisher in association with the New Zealand Society for Horticultural Science, New Zealand. pp. 420-428.

Brook, P. J. 1992. Botrytis Stem-end Rot and Other Storage Diseases of Kiwifruit - A Review. Proceedings Second International Symposium on Kiwifruit, February 1991, Palmerston North, New Zealand. *Acta Horticulturae* **297**: 545-550.

Bryant, T. G. 1991. Kiwifruit pollination: A synthesis of four year's endeavour. *The New Zealand Beekeeper* November pp. 7-11.

Bryant, T. G. 1986. The use of honey bees as pollinators of kiwifruit. *Kiwifruit Pollination Seminar Proceedings*, Ministry of Agriculture and Fisheries, October 1986, Tauranga, New Zealand, pp. 1-7.

Chou, M. C. and Preece, T. F. 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. *Annals of Applied Biology* **62**: 11-22.

Clark, C. J. 1990. Kiwifruit pollen - is it junk food for bees. *New Zealand Kiwifruit* **74**: 22.

Clark, C. J. and Lintas, C. 1992. Chemical composition of pollen from kiwifruit vines. *New Zealand Journal of Crop and Horticultural Science* **20**: 337-344.

Clinch, P. G. 1984. Kiwifruit pollination by honey bees 1. Tauranga observations, 1978-81. *New Zealand Journal of Experimental Agriculture.* **12**: 29-38.

Clinch, P. G. and Heath, A. C. G. 1985. Wind and bee pollination research. *New Zealand Kiwifruit* **14**: 15.

Coley-Smith, J. R. 1980. Sclerotia and other structures in survival. In: *The Biology of Botrytis*. Edited by Coley-Smith, J. R., Veroeff, K. and Jarvis, W. R. Academic Press Inc. (London) Ltd. pp. 85-114.

Coley-Smith, J. R., Veroeff, K. and Jarvis, W. R. 1980. *The Biology of Botrytis*. Academic Press Inc. (London) Ltd. 318 p.

Craig, J. L. and Stewart, A. M. 1988. A review of kiwifruit pollination: where to next? *New Zealand Journal of Experimental Agriculture* **16**: 385-399.

De Jong, M. D., Scheepens, P. C., and Zadoks, J. C. 1990. Risk analysis applied to biological control of a forest weed, using the Gaussian plume model. *Grana* **29**: 139-145.

De Wael, L., De Greef, M. and Van Laere, O. 1990. The honeybee as a possible vector of *Erwinia amylovora* (Burr.) Winslow *et al. Acta Horticulturae* **273**: 107-113.

Donovan, B. J. and Macfarlane R. P. 1984. Bees and Pollination. In: *New Zealand Pest and Beneficial Insects*. Edited by R. R. Scott. Lincoln University, New Zealand. pp. 247-270.

Dyer, F. C. and Seeley, T. D. 1989. Orientation and foraging in honeybees. In: *Insect Flight*. Edited by Goldsworthy, G. J. and Wheeler, C. H. CRC Press, Boca Raton. pp. 205-230.

Ellis, M. B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Agricultural Bureaux, Surrey, England. 608 p.

Elmer, P. A. G., Gaunt, R. E., Boyd-Wilson, K. S. H., Pyke, N. B., and Fermaud, M. 1995. External contamination of kiwifruit by *Botrytis cinerea*: an important source of inoculum for fruit infection. *Proceedings of the 48th New Zealand Plant Protection Conference,* 1995. pp. 95-100.

Elmer, P. A. G., Boyd-Wilson, K. S. H., Cook, D. W., Whelan, H. G., Pyke, N. B. and Gaunt, R. E. 1994. Sources of Botrytis in kiwifruit vines and spore contamination at harvest. *Proceedings of the 5th National Research Conference of the New Zealand Kiwifruit Marketing Board.* pp. 19-21.

Elmer, P. A. G., Boyd-Wilson, K. B. W., Cook, D., Pyke, N., Framptom, C. F. and Whelan, H. G. 1993. The epidemiology of *Botrytis cinerea* in Motueka kiwifruit orchards. Report to the New Zealand Kiwifruit Marketing Board. 41 p.

Elmer, P. A. G., Boyd-Wilson, K. B. W., Pyke, N., and Framptom, C. F. 1992. An investigation of sources of *Botrytis cinerea* inoculum and the relationship between conidial populations of *B. cinerea* and microclimate in Motueka orchards. *Report to the New Zealand Kiwifruit Marketing Board.* 48 p.

Erickson, E. H. 1975. Surface electric potentials on worker honeybees leaving and entering the hive. *Journal of Apicultural Research* **14**(3/4): 141-147.

Ferguson, A. R. 1984. Kiwifruit: A Botanical Review. In: *Horticultural Reviews* (Volume 6). Edited by Janick, J. AVI Publishing Company, Inc. Westport, Connecticut. pp. 1-64.

Ferguson, A. R. and Bollard, E. G. 1990. Domestication of the Kiwifruit. In: *Kiwifruit: Science and Management*. Edited by Warrington, I.J. and Weston, G.C. Ray Richards Publisher in association with the New Zealand Society for Horticultural Science, New Zealand. pp. 165-246.

Fermaud, M. and Gaunt, R.E. 1995. *Thrips obscuratus* as a potential vector of *Botrytis cinerea* in kiwifruit. *Mycological Research* **99**(3): 267-273.

Fermaud, M., Gaunt, R. E. and Elmer, P. A. G. 1994. The influence of *Thrips obscuratus* on infection and contamination of kiwifruit by *Botrytis cinerea*. *Plant Pathology* **43**: 953-960.

Fermaud, M. and Le Menn, R. 1989. Association of *Botrytis cinerea* with grape berry moth larvae. *Phytopathology*. **79**: 651-656.

Fitt, B. D. L. and McCartney, H. A. 1986. Spore Dispersal in Relation to Epidemic Models. In: *Plant Disease Epidemiology* (Volume I). Edited by Leonard, K. J. and Fry, W. E. Macmillan Publishing Company, New York. pp. 311-345.

Fitt, B. D. L., McCartney, H. A. and Walkate, P. J. 1989. The role of rain in dispersal of pathogen inoculum. *Annual Review of Phytopathology* **27**: 241-270.

Free, J. B., Paxton, R. J. and Waghchoure, E. S. 1991. Increasing the amount of foreign pollen carried by honey bee foragers. *Journal of Apicultural Research* **30**(3/4): 132-136.

Free, J.B. and Williams, I.H. 1972. The transport of pollen on the body hairs of honey bees (*Apis mellifera* L.) and bumblebees (*Bombus* spp.L.). *Journal of Applied Ecology* **9**: 609-661.

Goodwin, R. M. 1986a. Kiwifruit flowers: anther dehiscence and daily collection of pollen by honey bees. *New Zealand Journal of Experimental Agriculture* **14**: 449-452.

Goodwin, R. M. 1986b. Honey bee pollination of kiwifruit. *Kiwifruit Pollination Seminar Proceedings*, Ministry of Agriculture and Fisheries, October 1986, Tauranga, New Zealand pp.1-18.

Goodwin, R. M. and ten Houten, A. 1989. Maximising pollination in poor weather. *New Zealand Kiwifruit* **53**: 20-22.

Goodwin, R. M., and Perry, J.H. 1992. Use of pollen traps to investigate the foraging behaviour of honey bee colonies in kiwifruit orchards. *New Zealand Journal of Crop and Horticultural Science* **20**: 23-26.

Goodwin, R. M. and Steven, D. 1993. Behaviour of honey bees visiting kiwifruit flowers. *New Zealand Journal of Crop and Horticultural Science* **21**: 17-24.

Gregory, P. H. 1976. *Outdoor Aerobiology.* Oxford University Press, London. 16 p.

Gregory, P. H. 1973. *The Microbiology of the Atmosphere.* Second edition. Edited by Polunin, N. Leonard Hill Books, Bucks, Great Britain. 377 p.

Gross, H. R., Hamm, J. J., and Carpenter, J. E. 1994. Design and application of a hive-mounted device that uses honey bees (Hymenoptera: Apidae) to disseminate *Heliothis* nuclear polyhedrosis virus. *Environmental Entomology* **23**(2): 492-501.

Hallet, I. and Sharrock, K. 1993. Penetrating the picking scar's defences. *New Zealand Kiwifruit* **96**: 7-9.

Hallett, I., Sharrock, K., Pennycook, S. and Manning, M. 1991. Don't pack immediately after picking. *New Zealand Kiwifruit* **78**: 12-13.

Harris, K. F. 1981. Arthropod and nematode vectors of plant viruses. *Annual Review of Phytopathology* **19**: 391-426.

Harrison, F. W., Brewer, J. W. and Merril, L. D. 1980. Insect involvement in the transmission of bacterial pathogens. In: *Vectors of Plant Pathogens.* Edited by Harris, K.F. and Maramorosch, K. Academic Press, New York, USA. pp. 201-276.

Hartnill, W. F. T. 1975. Germination of *Botrytis* and *Sclerotinia* spores in the presence of pollen on tobacco. *New Zealand Journal of Agricultural Research* **18**: 405-407.

Henis, Y., Kenneth, R. and Barash, L. 1987. Survival and Dormancy of Fungi. pp. 169-266. In: *Survival and Dormancy of Microorganisms.* Edited by Henis, Y. John Wiley & Sons, Inc, USA.

Hennebert, G. L. and Weresub, L. K. 1979. Terms for states and forms of fungi, their names and types. In: *The Whole Fungus: The Sexual - Asexual Synthesis, Volume I.* Edited by Kendrick, B. National Museums of Canada. pp. 27-30.

Hill, S. A. 1984. *Methods in Plant Virology.* Blackwell Scientific Publications, London. 167 p.

Holland, P. T., McGhie, T. K. and Malcolm, C. P. 1987. Pesticide residue monitoring, 1980-1986 - a story of continuing progress. *New Zealand Kiwifruit* **29**: 24-25.

Holmes, F. W. 1980. Bark Beetles, *Ceratocystis ulmi* and Dutch Elm Disease. In: *Vectors of Plant Pathogens.* Edited by Harris, K.F. and Maramorosch, K. Academic Press, New York, USA. pp. 133-143.

Hopping, M. E. 1990. Floral biology, pollination, and fruit set. In: *Kiwifruit: Science and Management.* Edited by Warrington, I.J. and Weston, G.C. Ray Richards Publisher in association with the New Zealand Society for Horticultural Science, New Zealand. pp. 71-96.

Hoyte, S. M., Perry-Meyer, L. J. and Hill, R. A. 1994. Incidence of *Botrytis cinerea* and colonisation of necrotic leaf tissue in kiwifruit canopies. Research note, *Proceedings of the 47th New Zealand Plant Protection Conference*, 1994. pp. 356-358.

Huang, H. C., Richards, K. W. and Kokko, E. G. 1986. Role of the Leafcutter Bee in Dissemination of *Verticillium albo-atrum* in Alfalfa. *Phytopathology* **76**: 75-79.

Jarvis, W. R. 1980a. Taxonomy. In: *The Biology of Botrytis*. Edited by Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R. Academic Press, Inc. (London) Ltd. pp. 1-17.

Jarvis, W. R. 1980b. Epidemiology. In: *The Biology of Botrytis*. Edited by Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R. Academic Press, Inc. (London) Ltd. pp. 219-250.

Jarvis, W. R. 1977. Botryotinia and Botrytis Species: taxonomy, physiology and pathogenicity. Research Branch, Canada Department of Agriculture, Ottawa. 195 p.

Jay, D. and Jay, C. 1984. Observations of Honeybees on Chinese Gooseberries ('Kiwifruit') in New Zealand. *Bee World* **65**(4): 155-166.

Jennertsen, O. 1983. Butterflies as vectors of *Ustilago violacea* spores between caryophyllaceous plants. *Oikos* **40**: 125-130.

Johansen, C., Kious, C., Mayer, D. 1985. *Bee Research Investigations*. Washington State Universtiy. 38 pp.

Johnson, K. B., Stockwell, V. O., Burgett, D.M., Sugar, D. and Loper, J. E. 1993. Dispersal of *Erwinia amylovora* and *Pseudomonas fluorescens* by Honey Bees from Hives to Apple and Pear Blossoms. *Phytopathology* **83**: 478-484.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore trap. *Netherlands Journal of Plant Pathology* **96**: 247-250.

Knight, J. and Bourdot, G. W. The development of a selective medium for the isolation of *Sclerotinia sclerotiorum* ascospores. Manuscript in preparation.

Kohn, L. M. 1979. Delimitation of the economically important plant pathogenic *Sclerotinia* species. *Phytopathology* **69**(8): 881-886.

Korf, R. P. 1973. Discomycetes and tuberales. In: *The Fungi; An Advanced Treatise, Volume IV A*. Edited by Ainsworth, G. C., Sparrow, F. K., and Sussman, A. S. Academic Press, New York & London. pp. 249-319.

Lavey-Meir, G., Barkai-Golan, R. and Kopeliovitch, E. 1988. Initiation at the flowering stage of post-harvest Botrytis stem-end rot in normal and non-ripening tomato fruits. *Annals of Applied Biology* **112**: 393-396.

Long, P. G. and Bautista-Banos, S. 1994. Curing and infection of kiwifruit by *Botrytis cinerea*. *Proceedings of the 5th National Research Conference of the New Zealand Kiwifruit Marketing Board.* pp. 31-34.

Lyford, P. and Underwood, R. 1992. The quest for larger fruit. *New Zealand Kiwifruit* **95**: 11.

MacFarlane, R. P. and Ferguson, A. M. 1984. Kiwifruit pollination: a survey of the insect pollinators in New Zealand. *International Symposium of Pollinisation*, Versailes, September 1983. pp. 367-373.

Manning, M. A. and Brook, P. J. 1991. Growth of dicarboximide-resistant strains of *Botrytis cinerea* at low temperatures. *Australasian Plant Pathology* **20**(3): 101-102.

Manning, M. A. and Pak, H. A. 1993. Production of Botrytis rots. *New Zealand Kiwifruit* **96**: 22-23.

McCartney, H. A. 1994. Spore Dispersal: Environmental and Biological Factors. In: *Ecology of Plant Pathogens*. Edited by Blakeman, J. P. and Williamson, B. CAB International, UK. pp. 171-185.

McClellan, W. D. and Hewitt, W. B. 1973. Early Botrytis rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* **63**: 1151-1157.

McNicol, R. J. and Williamson, B. 1989. Systemic infection of black currant flowers by *Botrytis cinerea* and its possible involvement in premature abscission of fruits. *Annals of applied Biology* **114**: 243-254.

McNicol, R. J., Williamson, B. and Dolan, A. 1985. Infection of red raspberry styles and carpels by *Botrytis cinerea* and its possible role in post-harvest grey mould. *Annals of Applied Biology*. **106**: 49-53.

Nair, N. G. 1985. Fungi Associated with Bunch Rot of Grapes in the Hunter Valley. *Australian Journal of Agricultural Research* **36**: 435-442.

Opgenorth, D. C. 1983. Storage rot of California-grown kiwifruit. *Plant Disease* **67**: 382-383.

Pak, H. A. and Manning, M. A. 1993. Epidemiology of *Botrytis* on Kiwifruit - methods for quantifying the effect of orchard management practices on *Botrytis* populations. Report to the New Zealand Kiwifruit Marketing Board. 25 p.

Pedgley, D. 1982. *Windborne Pests and Diseases: Meteorology of Airborne Organisms.* Ellis Horwood Ltd, West Sussex, England. 241 p.

Peng, G., Sutton, J. C., and Kevan, P. G. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. *Canadian Journal of Plant Pathology*. **14**: 117-129.

Pennycook, S.R. 1989. *Plant diseases recorded in New Zealand, Volume II.* Department of Scientific and Industrial Research, Auckland, New Zealand. 502 p.

Pennycook, S. R. 1985. Fungal fruit rots of *Actinidia deliciosa* (kiwifruit). *New Zealand Journal of Experimental Agriculture* **13**: 289-299.

Pennycook, S. R. and Manning, M. A. 1992. Picking wound curing to reduce Botrytis storage rot of kiwifruit. *New Zealand Journal of Crop and Horticultural Science* **20**: 357-360.

Pennycook, S. R. and Manning, M. A. 1985. Minimize number of fungicide sprays. *New Zealand Kiwifruit* **15**: 17.

Poole, P. R. and McLeod, L. C. 1992. Inhibition of *Botrytis cinerea* infection in kiwifruit tissues. *Proceedings of the Second International Symposium on Kiwifruit*, February 1991, Palmerston North, New Zealand. *Acta Horticulturae* **297**: 599-603.

Pyke, N. B., Manktelow, D. G., Elmer, P. A. G. and Tate, K. G. 1994. Postharvest dipping of kiwifruit in iprodione to control stem-end rot caused by *Botrytis cinerea*. *New Zealand Journal of Crop and Horticultural Science* **22**: 81-86.

Root, A. I. 1983. The ABC and XYZ of Bee Culture. *AI Root Company*, Medina, Ohio, USA. 703 pp.

Roy, B. A. 1994. The use and abuse of pollinators by fungi. *Trends in Ecology and Evolution* **9**: 335-339.

Sale, P. R. 1985. *Kiwifruit Culture*. Edited by Ashenden Williams, D. Government Printing Office, New Zealand. 96 pp.

Sharrock, K. R. and Hallet, I. C. 1992. Physiology of Botrytis infection in kiwifruit. *Proceedings Second International Symposium on Kiwifruit*, February 1991, Palmerston North, New Zealand. *Acta Horticulturae* **297**: 551-557.

Shaw, D. E. 1990. The incidental collection of fungal spores by bees and the collection of spores in lieu of pollen. *Bee World* **71**(4): 158-176.

Shykoff, J. A. and Bucheli, E. 1995. Pollinator visitation patterns, floral rewards and the probability of transmission of *Microbotryum violaceum*, a venereal disease of plants. *Journal of Ecology* **83**:189-198.

Smith, R. 1993. Kiwigreen retains Italian market access. *New Zealand Kiwifruit* **99**: 22-23.

Smith, P. L. 1979. Splines as a useful and convenient statistical tool. *American Statistician* **33**: 57

Sommer, N. F. and Saudi, J. E. 1984. Postharvest disease and storage life of kiwifruit. *Acta Horticulturae* **157**: 295-302.

Thomson, S. V., Hansen, D. R., Flint, K.M., and Vandenburg, J.D. 1992. Dissemination of bacteria antagonistic to *Erwinia amylovora* by honey bees. *Plant Disease* **76**: 1052-1056.

Tomkins, A. R., Wilson, D.J., and Thomson, C. 1992. Thrip control with fluvalinate on kiwifruit. *Proceedings 45th New Zealand Plant Protection Conference* 1992. pp. 162-166.

Ward, S. A., Wagenmakers, P. S. and Rabbinge, R. 1989. Dispersal and dispersion in space. In: *Simulation and systems management in crop protection.* Edited by Rabbinge, R., Ward, S. A. and van Laar, H. H. Pudoc, Wageningen, the Netherlands. pp. 99-116.

Wiktelius, S. 1980. Aerial dispersal of aphids into Sweden. *The First International Conference on Aerobiology*, Munich, Germany, 13-15 August, 1978, pp. 220-226.

Winston, M. L. 1987. *The Biology of the Honey Bee*. Harvard University Press, London. 281 p.

APPENDIX I

V8 Juice Agar

Ingredients per 1 Litre:

Distilled H_20	800 ml
V8 Tomato Juice	200 ml
Bacteriological Agar	20 g
Calcium Carbonate	3.0 g

Preparation:
Add 200 ml V8 juice to lukewarm distilled water. Add calcium carbonate and the agar mixture. Heat to melt agar and sterilize.

Source: Sourcebook of Laboratory Exercises in Plant Pathology America. 1962. *Phytopathological Society* **67**: 270.

Kerssies Botrytis Selective Medium

Basal Medium (ingredients per 1 Litre):

Sodium Nitrate	1.0 g
di-Potassium hydrogen orthophosphate	1.2 g
Magnesium sulphate	0.2 g
Potassium chloride	0.15 g
Glucose	20.0 g
Agar	25.0 g
Triton N101 (wetting agent, small colonies)	5 ml

Additives (ingredients per 1 Litre):

Terrachlor (PCNB 75% WP)*	0.015 g
Maneb (M22 80% WP)*	0.01 g
Chloramphenicol**	0.05 g
Copper Sulphate	2.20 g
Rubigan (12% FENARIMOL)	0.1 ml
Tannic Acid*	5.0 g
Streptomycin sulphate*	10 ml
Ridomil 2EC	10 µl
5.0 N NaOH	

* extremely dangerous chemicals

Kerssies Botrytis-selective agar was either mixed and poured in bulk 8 litre batches using an automatic agar pourer (Technomat 125) or prepared in a fumehood, while wearing protective gloves and lab coat.

Preparation:
To prepare one litre, mix the basal ingredients in 800 ml water. Put a magnetic flea in with this and autoclave at 121 degrees C for 15 minutes.

Meanwhile measure out additives and additionals aseptically and mix with 200 ml cooled sterile water in a sterile flask with a sterile flea. Make sure additives are mixed properly and when the basal medium has cooled to around 65 degrees measure 200 ml of additives into each 800 ml of basal medium. Adjust pH to 4.5 with 5.0 N NaOH.

Source: Modification of **Kerrsies, A. 1990.** A selective medium for *Botrytis cinerea* to be used in a spore-trap. *Netherlands Journal of Plant Pathology* **96**: 247-250.

APPENDIX II

Raw data for Section 2.3: Mean number of *B. cinerea* cfu per honey bee foraging on pistillate kiwifruit flowers, staminate kiwifruit flowers, or manuka flowers at early, full and late stages of kiwifruit bloom.

Early flower (4/12/93)			Full flower (10/12/93)			Late flower (15/12/93)		
Pistillate	Staminate	Manuka	Pistillate	Staminate	Manuka	Pistillate	Staminate	Manuka
6	1	15	0	8	4	25	8	12
5	3	8	2	10	10	11	12	15
4	0	24	24	15	0	7	10	26
0	2	14	8	18	12	25	14	36
1	2	9	2	6	22	12	17	18
4	1	11	4	10	4	9	10	30
7	3	15	15	13	14	13	20	26
1	3	8	15	11	12	12	5	16
1	3	8	2	320	8	16	50	5
0	2	7	40	15	25	5	13	5
2	1	2	25	25	11	4	25	4
2	5	3	8	20	1	20	21	1
2	3	6	8	14	50	8	70	0
1	0	40	13	10	13	4	40	4
5	4	1	2	7	6	17	8	6
0	3	6	11	16	1	10	5	6
4	0	0	2	12	15	9	4	26
8	8	0	20	2	18	10	15	80
0	0	12	19	6	28	12	19	34
1	4	4	6	6	3	19	9	30

Raw Data for Section 3.3: Number of *B. cinerea* cfu picked up by individual honey bees foraging on staminate kiwifruit flowers. *B. cinerea* spores applied (plus spores) or not applied (minus spores) to flowers.

Plus Spores		Minus Spores	
Bees	Control	Bees	Control
2700	1	380	0
3100	9	50	0
2500	5	5	0
3500	15	25	0
1500	2	0	0
2800	13	0	0
3500	4	8	0
745	39	5	0
6200	0	0	0
6400	10	19	0

Plus Spores		Minus Spores	
Bees	Control	Bees	Control
9400	20	2	0
7100	2	1	0
4300	5	96	0
3900	15	1	0
6600	0	14	0
2800	0	1	0
5200	2	16	0
1400	24	2	0
7000	1	0	0
9000	9	62	0
1405	2		
6500	1120		
6000	0		
3700	0		
6700	1		
8300	3		
10600	5		
670	1		
7600	125		
5700	0		
745	0		
5800	0		
3600	3		
16200	1		
5100	0		
5100	0		
3500	4		
1500	1		
8100	0		
3000	11		
3600	2		
5200	3		
5000	2		
1500	0		
1600	0		
1400	19		
3500	0		
2500	94		
4400	4		
5300	7		
2000	0		
875	39		
1800	3		
260	0		
8500	2		

Calculation for comparison of shapes of log-linear and spline curves in Section 4.3:

$= {}^{(\Delta SS/\Delta df)}/_{\text{Residual Mean Square (spline)}} \approx F_{\Delta df,\ \text{residual df (spline)}}$

$= {}^{(54.9-51.6)\ /\ (2-1)}/_{0.4940} \approx F_{1,277}(5\%\ \text{level})$

$= 6.68 \approx 3.84$, i.e., regression equations are significantly different at the 5% level.

Raw data for Chapter 4:

Treatment (Trt) = 1 (honey bee), 2 (wind) and 3 (control)
Direction (dir) = North and South
Distance (dist) = distance (m) of sample from edge of inoculum zone
Average *B. cinerea* colony forming units (cfu) = average cfu per flower in each sample

		SITE A			SITE B			SITE C			SITE D			SITE E			SITE F	
Trt	**dir**	**dist**	**cfu**	**dir**	**dist**	**cfu**	**dir**	**dist**	**cfu**	**dir**	**Dist**	**cfu**	**dir**	**dist**	**cfu**	**dir**	**dist**	**cfu**
1	1	0.3	330	1	0.2	510	1	0.2	860	1	0.2	350	1	0.2	470	1	0.3	400
1	1	0.3	330	1	0.2	1100	1	0.2	610	1	0.3	380	1	0.3	240	1	0.4	210
1	1	0.3	570	1	0.3	620	1	0.2	570	1	0.4	410	1	0.3	690	1	0.4	820
1	1	0.4	420	1	0.4	760	1	0.3	300	1	0.4	350	1	0.3	410	1	0.5	440
1	1	0.4	180	1	0.4	450	1	0.3	830	1	0.5	800	1	0.4	580	1	0.6	200
1	1	0.5	230	1	0.5	380	1	0.3	2150	1	0.6	0	1	0.4	610	1	0.6	270
1	1	0.6	100	1	0.6	270	1	0.4	750	1	0.7	420	1	0.5	330	1	0.7	350
1	1	0.6	400	1	0.6	220	1	0.4	660	1	0.7	340	1	0.6	2500	1	0.7	260
1	1	0.7	270	1	1	260	1	0.6	1260	1	0.8	340	1	0.9	200	1	0.9	370
1	1	0.7	540	1	1.1	180	1	0.7	930	1	0.8	350	1	1.1	50	1	0.9	280
1	1	0.8	460	1	1.1	70	1	0.8	390	1	1	310	1	1.1	640	1	1	130
1	1	0.9	190	1	1.2	80	1	0.8	620	1	1	180	1	1.2	80	1	1	140
1	1	0.9	270	1	1.3	540	1	0.9	290	1	1	760	1	1.2	250	1	1.1	600
1	1	1	210	1	1.4	40	1	1	580	1	1.2	310	1	1.3	150	1	1.1	240
1	1	1	440	1	1.7	90	1	1	300	1	1.3	210	1	1.3	110	1	1.4	170
1	1	1.2	40	1	1.7	240	1	1.2	140	1	1.3	440	1	1.4	220	1	1.4	40
1	1	1.3	170	1	1.7	420	1	1.6	810	1	1.6	160	1	1.4	1000	1	1.6	230
1	1	1.7	220	1	1.8	50	1	1.7	630	1	1.7	150	1	1.7	240	1	1.8	100
1	1	1.7	230	1	1.8	160	1	1.8	220	1	1.8	120	1	1.8	300	1	1.9	300
1	1	1.8	170	1	1.8	180	1	1.9	250	1	1.8	400	1	1.9	90	1	1.9	240
1	1	1.8	100	1	1.9	180	1	2	100	1	1.8	400	1	1.9	220	1	2	60
1	1	1.9	120	1	2	160	1	2	330	1	1.9	20	1	2	870	1	2	370
1	1	1.9	100	1	2.2	180	2	0.2	1290	1	1.9	140	1	2	170	2	0.2	580
1	1	2	200	2	0.2	520	2	0.2	1250	1	2	240	1	2.1	140	2	0.4	400
1	1	2	60	2	0.3	600	2	0.3	730	1	2	220	1	2.1	620	2	0.4	100
1	2	0.1	730	2	0.4	370	2	0.3	480	1	2.1	170	2	0.1	700	2	0.5	550
1	2	0.2	600	2	0.4	190	2	0.3	3400	2	0.2	180	2	0.3	2070	2	0.5	100
1	2	0.2	820	2	0.4	210	2	0.4	440	2	0.3	360	2	0.3	340	2	0.6	500
1	2	0.3	250	2	0.5	280	2	0.5	480	2	0.3	110	2	0.4	880	2	0.5	640
1	2	0.3	430	2	0.5	920	2	0.6	100	2	0.3	450	2	0.5	460	2	0.9	190
1	2	0.3	290	2	0.5	300	2	1	210	2	0.4	560	2	0.5	390	2	0.9	450
1	2	0.4	180	2	0.6	620	2	1	110	2	0.4	460	2	0.6	680	2	1	180
1	2	0.4	600	2	0.6	310	2	1	330	2	0.4	1320	2	0.9	269	2	1.1	230
1	2	1	250	2	1	160	2	1.1	350	2	0.6	730	2	1	430	2	1.1	110
1	2	1	230	2	1	320	2	1.1	900	2	0.7	240	2	1	300	2	1.4	160

		SITE A			SITE B			SITE C			SITE D			SITE E			SITE F	
Trt	dir	dist	cfu	dir	dist	cfu	dir	dist	cfu	dir	Dist	cfu	dir	dist	cfu	dir	dist	cfu
1	2	1.1	190	2	1	440	2	1.1	120	2	0.8	650	2	1.1	150	2	1.6	710
1	2	1.2	110	2	1.1	450	2	1.2	800	2	1	100	2	1.3	290	2	1.7	80
1	2	1.3	180	2	1.2	630	2	1.3	80	2	1	760	2	1.3	430	2	1.7	50
1	2	1.3	190	2	1.2	420	2	1.5	160	2	1	30	2	1.5	330	2	1.9	170
1	2	1.3	210	2	1.3	90	2	1.5	170	2	1.1	40	2	1.6	150	2	2	380
1	2	1.5	130	2	1.3	140	2	1.7	200	2	1.6	70	2	1.7	580	2	2	120
1	2	1.8	70	2	1.6	180	2	1.7	360	2	1.7	150	2	1.7	90	2	2.1	50
1	2	1.8	120	2	1.6	240	2	1.8	250	2	1.8	90	2	1.7	80			
1	2	1.9	240	2	1.7	640	2	1.8	260	2	1.8	260	2	1.8	80			
1	2	1.9	420	2	1.7	390	2	2	150	2	1.9	220	2	2	30			
1	2	2	180	2	2.1	120	2	2.1	240	2	1.9	140	2	2	120			
1	2	2	200	2	2.2	290				2	2	50	2	2.2	70			
1	2	2.1	80	2	2.3	120				2	2.1	120						
1										2	2.1	540						
2	1	0	6	1	0	4	2	0	17	1	0	0	2	0	3	2	0	0
2	1	0	17	1	0	5	2	0	30	1	0	6	2	0	19	2	0	0
2	1	0	0	1	0	4	2	0	0	1	0	2	2	0	7	2	0	10
2	1	0.2	1	1	0.2	0	2	0.2	7	1	0.1	3	2	0.4	2	2	0.4	0
2	1	0.3	0	1	0.2	3	2	0.8	3	1	0.3	2	2	0.5	0	2	0.6	0
2	1	0.8	0	1	0.3	0	2	0.8	2	1	1.2	2	2	0.5	1	2	0.7	2
2	1	0.8	0	1	0.6	2	2	1	1	1	1.3	1	2	0.5	0	2	0.7	0
2	1	1	0	1	0.6	3	2	1.9	0	1	1.3	0	2	0.9	8	2	1.2	0
2	1	1	1	1	1.2	2	2	2	3	1	1.6	35	2	0.9	5	2	1.9	0
2	1	1.3	0	1	1.2	0	2	2	1	1	1.6	0	2	1.2	1	2	2.2	0
2	1	1.7	0	1	1.6	1	2	2.1	1	1	1.7	0	2	1.7	0			
2	1	1.8	0	1	1.6	1				1	1.7	0	2	1.7	0			
2	1	2	10	1	1.7	0							2	1.9	0			
2				1	1.8	0												
3	1	0	0	1	0	0	2	0	0	1	0	0	2	0	0	2	0	0
3	1	0	0	1	0	1	2	0	0	1	0	0	2	0	0	2	0	0
3	1	0	0	1	0	0	2	0	0	1	0	1	2	0	5	2	0	0
3	1	0.5	0	1	0.2	0	2	0.4	0	1	0.2	0	2	0.1	0	2	0.4	0
3	1	0.5	0	1	0.2	0	2	0.4	0	1	0.5	0	2	0.5	2	2	0.4	0
3	1	0.6	3	1	0.3	0	2	0.8	0	1	1.2	0	2	1	0	2	1.1	0
3	1	1.1	0	1	1.3	0	2	0.8	0	1	1.2	0	2	1.2	0	2	1.3	0
3	1	1.2	0	1	1.4	0	2	1	1	1	1.3	0	2	1.2	0	2	1.4	1
3	1	1.4	0	1	2.3	0	2	1.6	0	1	1.9	0	2	2	0	2	1.9	0
3	1	1.8	0				2	1.7	0	1	1.9	0	2	2.1	0	2	2	0
3	1	1.8	0				2	1.7	0	1	2	2	2	2.2	0	2	2	0
3	1	1.9	0															

Raw data for Chapter Five: Mean *B. cinerea* cfu per fruit. *B. cinerea* spore application treatments 1 - 4 (0, $1x10^4$, $1x10^5$ and $1x10^6$ spores per ml, respectively).

		Mean *B. cinerea* cfu per fruit		
Plot	Spore treatment	Petal fall	Mid fruit	Mature fruit
1	1	267	2667	11970
2	1	533	7333	1774
3	1	1733	1600	2120
4	1	267	2133	6206
5	1	107	18667	1448
1	2	800	5333	7980
2	2	9200	1600	19280
3	2	2000	29333	9950
4	2	173	9947	1092
5	2	10333	17333	16000
1	3	747	6400	6250
2	3	4400	18667	1600
3	3	1333	7200	10326
4	3	9067	30667	5614
5	3	8800	36267	6916
1	4	42667	6933	17290
2	4	31467	33333	9310
3	4	49067	25413	13314
4	4	22000	14667	36354
5	4	22667	94667	5154